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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

Memorandum

Subject: EPA Id No.: 059102. Chlorpyrifos methyl: Review of the 1988 mouse carcinogenicity, 1992 rat developmental toxicity study and the 1990 subchronic oral toxicity study in dogs.

PC Code: 059102
DP Barcode No.: D250872
Submission No.: S551248

From: John Doherty *John Doherty* 5/28/99
ReRegistration Branch III
Health Effects Division 7509C

To: Mark Hartman
Product Manager Team 52
Special Review and ReRegistration Division 7507C

Through: Steve Knizner *Steve Knizner*
Branch Senior Scientist
ReRegistration Branch III
Health Effects Division 7509C

Attached are the completed DERs for the 1988 mouse carcinogenicity study (MRID No.: 44680602), the 1992 rat developmental toxicity study (MRID No.: 44680603) and dog subchronic feeding study (MRID NO.: 44680601). These studies were incorporated in the HIARC report for chlorpyrifos methyl dated May 17, 1999. A Copy of this HIARC report is available on the LAN.

DATA EVALUATION REPORT

CHLORPYRIFOS-METHYL

013399

STUDY TYPE: ONCOGENICITY FEEDING - MOUSE (83-2b)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 99-13

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JAN 09 1999

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Chlorpyrifos methyl

Oncogenicity Study (83-2b)

EPA Reviewer: J. Doherty, Ph.D.

Registration Action Branch 2

EPA Work Assignment Manager: S. Diwan, Ph.D.

Toxicology Branch I

_____, Date 6/1/97

_____, Date 6/1/97

DATA EVALUATION RECORD

STUDY TYPE: Oncogenicity Feeding - Mouse
OPPTS 870.4200 [§83-2b]

DP BARCODE: D250872

SUBMISSION CODE: S551248

P.C. CODE: 059102

TOX. CHEM. NO.:

TEST MATERIAL (PURITY): Chlorpyrifos methyl (purity, 97.4% a.i.)

SYNONYMS: O,O-dimethyl-[0-(3,5,6-trichloro-2-pyridyl)]phosphorothioate

CITATION: Yoshida, A., et al. (1988) Chlorpyrifos methyl: 18-month oral chronic toxicity and oncogenicity study in mice. The Institute of Environmental Toxicology, Suzuki-cho 2-772, Kodaira-shi, Tokyo 187, Japan, Laboratory study ID: GHF-R-166, MRID 44680602. Unpublished.

Yoshida, et al. (1985) Chlorpyrifos-methyl: 28:day oral toxicity study in mice. The Institute of Environmental Toxicology, Suzuki-cho 2-772, Kodaira-shi, Tokyo 187, Japan, Laboratory study ID: GHF-R-80, MRID 44668202. Unpublished.

SPONSOR: Dow Chemical Company, Midland, Michigan 48674; Dow Chemical Japan, Ltd., Uchisaiwai-cho 2-1-4, Chiyoda-ku, Tokyo 100, Japan

EXECUTIVE SUMMARY: In an oncogenicity study (MRID 44680602), Chlorpyrifos methyl (97.4% a.i., lot no. AGR 219561) was administered to groups of 52 male and 52 female pathogen free ICR Crj:CD-1® mice in the diet at concentrations of 0, 1, 5, 50, or 500 ppm for up to 78 weeks. These concentrations resulted in a nominal compound intake for each concentration level of 0.0816, 0.418, 4.40, and 44.0 mg/kg/day for males; 0.0815, 0.403, 3.94, and 41.5 mg/kg/day for females for 1 ppm, 5 ppm, 50 ppm, and 500 ppm dietary mixtures, respectively. Satellite groups containing 44 mice per sex per group were fed the same diets for 26 and 52 weeks.

Treatment related effects were noted at 500 ppm only. The mean **body weights** of the 500 ppm group males were decreased by 12% at 52 weeks and 17% at 78 weeks. Food consumption was slightly decreased in the 500 ppm dose group males during the first 12 weeks of the study, and overall food efficiency of the 500 ppm group males was lower (control 1.2; 500 ppm, 1.0, N.S.).

The total blood **cholesterol** was increased in 500-ppm group males by 39% ($p < 0.05$) compared to the control at 26 weeks and in 500-ppm group females by 45-79%, ($p < 0.05$ or 0.01) at all time points.

Increased incidence of **fatty changes in centrilobular hepatocytes** were seen in males killed at 52 weeks (500 ppm 75%; 25% of controls, $p < 0.01$), in main study males (500 ppm, 40%; controls, 18%, $p < 0.01$), in females killed at 52 weeks (500 ppm, 71%, controls, 4% $p < 0.01$), and in main study females (500 ppm, 40%; 6% controls, $p < 0.01$). A significantly increased incidence of **kidney tubular atrophy** was seen in main dose males (500 ppm, 60%; controls, 34%, $p < 0.01$). The incidence of kidney tubular atrophy was marginally but not statistically significantly increased at 50 ppm ($p = 0.08$) in the main study males. **Swelling of adrenal cortical cells** occurred in 42% ($p < 0.01$) of main study and 25% ($p < 0.01$) of 52 week interim sacrifice male mice.; this lesion did not occur in any animals fed lower doses or the control groups. **The LOAEL for systemic effects is 500 ppm for both sexes (44 mg/kg/day for males and 41.5 mg/kg/day for females) based on histopathological lesions in the liver, kidney and adrenal glands of male mice and in the liver of females mice. The NOAEL is 50 ppm (4.40 mg/kg/day for males and 3.94 mg/kg/day for females).**

Cholinesterase from plasma and red blood cells was moderately inhibited at 50 ppm by 47-70% ($p < 0.01$ or 0.05) in male mice and by 31-75% ($p < 0.01$ or 0.05) in female mice and severely inhibited by 500 ppm by 93-96% ($p < 0.01$) in male mice and by 87-97% ($p < 0.01$) in female mice at all time points (26, 52 and 78 weeks). At 50 ppm, brain cholinesterase activity was statistically decreased in males (14%, $p < 0.05$) only at week 78, and in females mice, a statistical decrease (25%, $p < 0.01$) was seen only at week 52. Brain cholinesterase was significantly decreased by 53-64% ($p < 0.01$) in 500 ppm group male mice and by 45-50% ($p < 0.01$) in 500 ppm group female mice at all time points. **The LOAEL for inhibition of cholinesterase is 50 ppm for both sexes (4.40 mg/kg/day for males and 3.94 mg/kg/day for females). The NOAEL is 5 ppm (0.418 mg/kg/day for males and 0.403 mg/kg/day for females).**

Treatment for up to 78 weeks with Chlorpyrifos methyl did not result in a significant increase in the incidence of neoplastic lesions at any site. The animals were adequately dosed as evidenced by decreased cholinesterase activity at 50 ppm and treatment-related microscopic lesions in both sexes at 500 ppm.

This oncogenicity study in the mouse is **Acceptable** and does satisfy the guideline requirement for an oncogenicity study (83-2b) in mice.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Chlorpyrifos methyl

Synonym: O,O-dimethyl-[0-(3,5,6-trichloro-2-pyridyl)]phosphorothioate

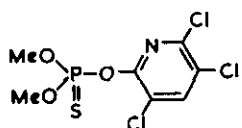
Description: amber crystalline solid

Lot/Batch #: Lot no. AGR 219561

Purity: 97.4%

Stability of compound: stable under normal storage conditions

CAS #:



2. Vehicle and/or positive control

The test material was mixed with feed; a positive control was not included in this study.

3. Test animals: Species: mouse

Strain: pathogen free ICR Crj:CD-1®

Age and weight at study initiation: age: 5 weeks; group mean weight: males: 26.0 g; females: 24.0 g

Source: Charles River Japan, Inc., Shimofurusawa, Atsugi-shi, Kanagawa

Housing: animals were housed 4 mice (same sex) per cage at study initiation.

Diet: Pulverized diet M, Oriental Yeast Co., Ltd., Azusawa, Itabashi-ku, Tokyo, *ad libitum*

Water: tap water, *ad libitum*

Environmental conditions: Temperature: $24 \pm 1^\circ\text{C}$

Humidity: $55 \pm 10\%$

by decreased cholinesterase activity at 50 ppm and treatment-related microscopic lesions in both sexes at 500 ppm.

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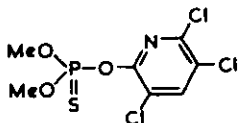
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Diet: Pulverized diet M, Oriental Yeast Co., Ltd., Azusawa, Itabashi-ku, Tokyo, *ad libitum*

Water: tap water, *ad libitum*

Environmental conditions: Temperature: 24 ± 1 °C

Humidity: 55 ± 10%

Air changes: 12/hour
 Photoperiod: 14 hours light/10 hours dark
 Acclimation period: 7 days for males, 12 days for females

B. STUDY DESIGN:

1. In life dates:

Start: December 5, 1985; End: Males: June 12; females: June 17, 1988

2. Animal assignment

Animals were assigned to the test groups in Table 1 by a method that ensured animals with similar weights were randomly distributed in all groups. Satellite groups were included for interim clinical tests and pathological examinations after 26 and 52 weeks of treatment.

TABLE 1. Study design					
Test group	Dietary concentration (ppm)	Dose to animals* (mg/kg/day)		Number of animals	
		Male	Female	Male	Female
1 main	0	0	0	52	52
2 main	1	0.0816	0.0815	52	52
3 main	5	0.418	0.403	52	52
4 main	50	4.40	3.94	52	52
5 main	500	44.0	41.5	52	52
1 sat.	0	—	—	44	44
2 sat.	1	—	—	44	44
3 sat.	5	—	—	44	44
4 sat.	50	—	—	44	44
5 sat.	500	—	—	44	44

Data taken from p. 10, Table 9, p. 55, and Table 10, p. 56, MRID 44680602.

*Daily dietary Chlorpyrifos methyl consumption was calculated from the mean weekly food consumption and body weight data and was based on nominal dietary levels of Chlorpyrifos methyl.

3. Dose selection:

The dose selections were based on a previous 28-day feeding study (laboratory study ID: GHF-R-80, MRID 44668202) utilizing specific pathogen-free ICR Crj:CD-1[®] mice, which was performed in the Institute of Environmental Toxicology laboratory. No-observed-adverse-effect-levels (NOAEL) of 5 ppm (0.651 mg/kg/day) for males

and 1 ppm (0.141 mg/kg/day) for females were determined. Lowest-observed-adverse-levels (LOAEL) of 10 ppm (1.27 mg/kg/day) for males based on decreased plasma and red blood cell cholinesterase activity and 5 ppm (0.745 mg/kg/day) for females based on decreased plasma cholinesterase activity were determined. Cholinesterase activity was decreased by 71-100% in plasma, red blood cells, and brain in all animals compared to the controls at 1000 ppm, and all animals died before treatment day 9 at 10,000 ppm. Details of the study are included in Appendix 1.

4. Diet preparation and analysis

Test diets were prepared 2-3 times a week by dissolving the test compound in acetone and mixing the resulting solution with an appropriate amount of basal diet in a mixer. Stability and homogeneity of the dietary mixtures were previously shown in a 28-day range-finding study (GHF-R-80; MRID 44668202). Samples of 1, 5, 10, 1000, and 10,000 ppm preparations were taken from the top, middle, and bottom of the mixing container to test for homogeneity. Samples of 10 ppm mixtures were stored at room temperature for 3, 7 and 14 days to test for stability. All dietary concentrations in the present study were analyzed for Chlorpyrifos methyl content monthly during the treatment period.

Results

Homogeneity: The coefficient of variability of samples of the dietary mixture taken from the top, middle and bottom of the mixer ranged from 2.7% for the 1 ppm mixture to 0.6% for the 1000 and 10,000 ppm mixtures. In this case, however, the test substance was not dissolved in acetone prior to mixing with the basal diet as it was in the present study. It would be expected that dissolving the chemical in acetone prior to mixing would result in an improvement in homogeneity.

Stability: Dietary mixtures containing 10 ppm Chlorpyrifos methyl stored for 3 days at room temperature were shown to be 97% of the initial assayed concentration of the freshly prepared mixture after storage and about 91% after 14 days storage.

Concentration analysis The routine concentration analyses of the 1 ppm dietary concentration showed agreement within 15% of the target concentration with 1 exception in 19 samples. The exceptions was 82% of the target concentration. The overall mean was 94% of the nominal concentration. The 5 ppm concentration agreed within 15% of the target concentration with 3 exceptions which were 80-83% of the nominal concentration. The overall mean agreed within 91% of the target 5 ppm concentration. The overall mean assayed concentrations of the 50 and 500 ppm mixtures agreed within 95% and 94%, respectively, of the target concentrations.

The dietary mixture preparation procedures were shown to be acceptable.

5. Statistics

Body weight, food consumption, water consumption, hematology, blood chemistry, cholinesterase, and absolute and relative organ weights were subjected to parametric analysis of variance (ANOVA). If a statistically significant difference was found among the groups, Dunnett's t-test or Scheffe's multiple comparison test was applied to identify statistically significant differences between treatment groups and their controls. When group variances were heterogeneous, the data were evaluated by Kruskal-Wallis nonparametric ANOVA. Bartlett's test was used to evaluate equality of variances.

Mann-Whitney's U test was applied to urinalysis data; clinical signs, mortality, and the incidences of pathological lesions were analyzed with Fisher's exact test.

Comparisons were considered statistically significant at $p < 0.05$.

C. METHODS:

1. Observations:

All animals were inspected daily for signs of toxicity and mortality and were given a detailed examination once each week.

2. Body weight

All animals were weighed at weekly intervals for the first 13 weeks and monthly thereafter.

3. Food and water consumption and compound intake

The mean daily food and water consumption for each animal in the main study was determined weekly during the first 13 weeks of treatment and monthly thereafter. This was accomplished by dividing the total food and water consumption for the cage by the number of animals in the cage and by the number of days being measured. The group mean food efficiency (mean body weight gained/100 g food consumed) at each weighing interval was calculated for animals in the main study. The compound intake (mg/kg/day) was calculated for each concentration from the food intake and body weight data.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations are not required and were not performed.

5. Blood was collected from the posterior vena cava of 10 satellite mice per sex per group after 26 and 52 weeks and from 10 main study mice per sex per group at 78

weeks for hematologic and serum chemistry evaluations. The mice were anesthetized with ether and were not fasted. Heparin was used as an anticoagulant. Blood smears for the differential leukocyte counts were stained with May-Grunwalds and Giemsa stains. The CHECKED (X) parameters were examined.

a. Hematology

<u>X</u>		<u>X</u>	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements		Red and white blood cell and platelet morphology
	(Thromboplastin time)		Red cell distribution width (RCDW)
	(Clotting time)		
	(Prothrombin time)		

* Minimum requirement for oncogenicity studies unless effects are observed, based on Subdivision F Guidelines.

b. Clinical chemistry

<u>X</u>		<u>X</u>	
X	Alkaline phosphatase (AIP)	X	Glucose
X	Glutamic oxaloacetic transaminase (GOT)	X	Blood urea nitrogen (BUN)
X	Glutamic pyruvic transaminase (GPT)	X	Total cholesterol (T.Chol)
X	Total protein (TP)	X	Calcium

c. Cholinesterase activity

Cholinesterase activity in red blood cells, plasma, and brain was evaluated in 10 animals per sex per dose at weeks 26, 52, and 78.

6. Urinalysis

Urinalysis was performed on 10 satellite mice per sex per group at 26 and 52 weeks and on 10 main study mice per sex per group at 78 weeks. Urine was collected by pressing on the abdomen prior to taking blood samples for hematology on the same mice.

<u>X</u>		<u>X</u>	
X	Specific gravity	X	Ketones
X	pH	X	Occult blood
X	Protein	X	Urobilinogen
X	Glucose		

7. Sacrifice and pathology

Necropsies were done on all animals in the main and satellite groups killed at the scheduled periods and on all animals that died or were killed at unscheduled times during the treatment period. The mice were killed by exsanguination under ether anesthesia. The CHECKED (X) tissues from all groups were collected for histopathological examination. Tissue samples were fixed in neutral-buffered 10% formalin. Preparations were primarily stained with hematoxylin and eosin. All tissues from animals in the control and 500 ppm satellite groups killed at 52 weeks and all animals in the main study were examined with light microscopy. The (XX) organs from all animals were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain**
	Oral tissue	XX	Heart*	X	Periph. nerve*
X	Salivary glands*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Esophagus*	X	Lymph nodes*	X	Pituitary*
X	Stomach*	X	Spleen*	X	Eyes*
X	Duodenum*	X	Thymus*		
X	Jejunum*				
X	Ileum*				
X	Cecum*	XX	UROGENITAL	XX	GLANDULAR
X	Colon*	X	Kidneys**	X	Adrenal gland*
X	Rectum*	XX	Urinary bladder*	X	Lacrimal/Harderian glands
XX	Liver**	X	Testes**	X	Mammary gland*
X	Gall bladder*	X	Epididymides	X	Parathyroids*
X	Pancreas*	X	Prostate	X	Thyroids*
		X	Seminal vesicle		Auditory sebaceous gland (Zymbal's gland)
		X	Coagulating gland		
		X	Preputial gland		
	RESPIRATORY	X	Ovaries*	X	OTHER
X	Trachea*	X	Uterus*	X	Bone*
X	Lung*		Cervix	X	Skeletal muscle*
	Nose		Oviduct	X	Skin* and subcutis
	Pharynx		Vagina		Mediastinal tissue
	Larynx				Mesenteric tissue
				X	All gross lesions and masses*

* Required for oncogenicity studies based on Subdivision F Guidelines.

* Organ weight required in oncogenicity studies.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

No significant treatment-related clinical signs were seen during the daily or weekly examinations. There was a slight increase in the frequency of decreased locomotor activity in males at the high dose compared to the control (control, 2%; 500 ppm, 10%), but the difference was not statistically significant.

2. Mortality

The percent survival at selected times during the study is given in Table 2. There were no significant trends or differences in survival of treated males or females compared to the control group. Both sexes had slightly better survival at 500 ppm than in the controls, but there were no significant differences.

TABLE 2. Percent survival of male and female mice fed Chlorpyrifos methyl for 78 weeks					
Weeks of study	Dietary concentration (ppm)				
	0	1	5	50	500
Males (n = 52)					
Week 52	87	90	94	90	88
Week 78	62	67	67	62	73
Females (n = 52)					
Week 52	98	87	87	92	96
Week 78	71	63	73	71	75

Data taken from Table 3, pp. 43-44, and Table 4, pp 45-46, MRID 44680602.

B. BODY WEIGHT

The group mean body weights and the calculated weight gain in male and female mice over selected time periods during treatment are summarized in Table 3. The body weights of males at 500 ppm were slightly decreased by up to about 8% throughout the study; however, the difference was statistically significant ($p < 0.05$) only 4 times in 29 measurements. The body weight gain in high-dose males was also decreased up to about 17% throughout most of the study. Body weights in high-dose females were decreased up to about 8% early in the study (week 11, $p < 0.01$), but by week 52, the body weights and body weight gain of high-dose females were comparable to the control group.

TABLE 3. Group mean body weights and body weight gains in male and female mice fed Chlorpyrifos methyl for up to 105 weeks (g)

Weeks of study	Dietary concentration (ppm)				
	0	1	5	50	500
Males					
Body weight at wk. 0	26.0 ± 1.5 ^a	26.0 ± 1.5	26.0 ± 1.4	26.0 ± 1.5	26.0 ± 1.5
Body weight at wk. 52	51.5 ± 6.4	52.1 ± 6.5	51.9 ± 5.5	51.3 ± 5.1	48.5 ± 5.6
Body weight at wk. 72	51.8 ± 6.3	52.5 ± 5.0	52.6 ± 6.2	51.3 ± 6.4	47.6 ± 5.2*
Body weight at wk. 76	51.2 ± 6.3	51.5 ± 5.6	52.6 ± 6.5	50.7 ± 7.0	46.9 ± 4.9
Weight gain at wk. 52 ^b	25.5	26.1	25.9	25.3	22.5
Weight gain at wk. 76 ^b	25.2	25.5	26.6	24.7	20.9
Females					
Body weight at wk. 0	24.0 ± 1.4	24.0 ± 1.3	24.0 ± 1.4	24.0 ± 1.3	24.0 ± 1.4
Body weight at wk. 11	36.7 ± 5.0	36.5 ± 4.9	36.3 ± 4.6	36.3 ± 4.2	33.8 ± 4.5**
Body weight at wk. 52	50.7 ± 6.4	50.2 ± 8.9	52.3 ± 6.4	52.9 ± 6.1	50.3 ± 7.9
Body weight at wk. 76	49.1 ± 7.3	50.1 ± 7.5	51.3 ± 6.7	52.9 ± 7.7	50.1 ± 8.5
Weight gain at wk. 52 ^b	26.7	26.2	28.3	28.9	26.3
Weight gain at wk. 76 ^b	25.1	26.1	27.3	28.9	26.1

Data taken and calculated from Table 5, pp. 47-48, and Table 6, pp.49-50, MRID 44680602

^aMean ± standard deviation

^bWeight gain was calculated by the reviewer

* p < 0.05, **p < 0.01, Significantly different from control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

The group mean daily food consumption for males at 500 ppm was slightly lower than the control for the first 12 weeks of the study (decreased by 10-12% in 6/12 measurements compared to the control, p < 0.05 or < .01). The food consumption at the high dose was similar to the control group for the remainder of the study, and the overall average daily food consumption values in males were similar in all groups (4.1, 4.0, 4.1, 4.2, and 4.0 g/mouse/day for control, 1, 5, 50, and 500 ppm, respectively, NS). Food consumption in females was comparable to controls for all treated groups throughout the study, and the overall average daily food consumption for females at 500 ppm was comparable to the control (3.5, 3.6, 3.6, 3.6, and 3.6 g/mouse/day, for control, 1, 5, 50, and 500 ppm, respectively, NS) (see Table 7, pp.51-52 and Table 8, pp. 53-54, MRID 44680602).

2. Compound consumption

Compound consumption was calculated by the study authors from the food consumption and body weight data. The results are given in Table 1.

3. Food efficiency

The overall mean food efficiency in males was slightly lower at the high dose compared to the control 1.2, 1.2, 1.2, 1.1, and 1.0 for control, 1, 5, 50, and 500 ppm, respectively, NS). In females, the mean food efficiency values were similar in all groups (1.4, 1.4, 1.4, 1.5, and 1.4 for control, 1, 5, 50, and 500 ppm, respectively, N.S.) (See Table 11, p. 57 and Table 12, p. 58, MRID 44680602).

4. Water consumption

Water consumption in male mice was slightly higher in the 5 and 50 ppm groups and slightly lower at 500 ppm than the control group throughout most of the study. The overall mean water consumption values were: control, 5.8; 1 ppm, 5.6; 5 ppm, 6.5; 50 ppm 6.6; and 500 ppm, 5.4 mL/mouse/day (N.S.) (see Table 13, pp. 59-60, MRID 44680602). Water consumption was decreased in females at 50 and 500 ppm compared to the control group. The decreased consumption at 500 ppm compared to the control group was statistically significant ($p < 0.05$ or < 0.01) during 8/11 weekly measurements (decreased by 13-18% compared to the control). The overall mean water consumption values for females were: control, 5.7; 1 ppm, 5.6; 5 ppm, 5.9; 50 ppm, 5.0; and 500 ppm, 4.9, (NS) (see Table 14, pp. 61-62, MRID 44680602).

5. Ophthalmoscopic examination

Ophthalmoscopic examination is not required in oncogenicity studies and was not done.

6. Urinalysis

Urine protein was lower in all treated male groups at 78 weeks than in the control group (number of animals with severe (++++) levels of protein: 7/10, 1/10, 1/10, 0/10, and 0/10 at 0, 1, 5, 50, and 500 ppm, respectively, $p < 0.05$ or 0.01 for all treated groups). Specific gravity was significantly ($p < 0.01$) elevated in 500-ppm group males and the pH was significantly decreased in 50- ($p < 0.01$) and 500-ppm ($p < 0.05$) group males at 52 weeks. The specific gravity of the urine in females was statistically higher than the control at 50 and 500 ppm (control, 1.067; 50 ppm, 1.095; 500 ppm, 1.099; $p < 0.05$) after 26 weeks of treatment and after 78 weeks (control, 1.050; 50 ppm, 1.079; 500 ppm, 1.080; $p < 0.01$). Data taken from Table 15, pp. 63-65 and Table 16, pp. 66-68, MRID 44680602.

D. BLOOD WORK1. Hematology

No significant treatment-related changes in hematologic parameters were seen in the study. Wide variations in the white blood cell (WBC) and unclassified cell (UC) counts were seen in males at 500 ppm and in the control (WBC count $10^3/\text{mm}^3$: 142.7 ± 437.6 vs 3.9 ± 2.2 for controls, NS; UC count $10^3/\text{mm}^3$: 139.0 ± 438.9 vs 0.1 ± 0.1 for controls, NS) and in 500 ppm female groups and the controls (WBC counts $10^3/\text{mm}^3$: 24.9 ± 71.6 vs 60.1 ± 124.8 for controls, NS; UC count $10^3/\text{mm}^3$: 22.0 ± 69.4 vs 24.6 ± 65.0 for controls, NS) after 78 weeks of treatment. Data taken from Table 17, pp. 69-74 and Table 18, pp. 75-80, MRID 44680602.

2. Clinical chemistry

Selected blood clinical chemistry values are given in Table 4. The total mean cholesterol was increased in males by 39% ($p < 0.05$) at 500 ppm compared to the controls after 26 weeks of treatment and by about 41% (NS) at 500 ppm after 52 weeks; however, the cholesterol level was comparable to the control after 78 weeks. Cholesterol values were increased by 45% ($p < 0.05$) in high-dose females at 26 weeks, by 79% at 52 weeks, and by 53% ($p < 0.01$) at 78 weeks of treatment compared to the control group. Alkaline phosphatase activity was statistically significantly decreased by 47% ($p < 0.05$) in high-dose females compared to the controls after 78 weeks of treatment; changes after 26 (-4%) and 52 weeks (+22%) were not statistically significant.

TABLE 4. Blood chemistry values in male and female mice fed Chlorpyrifos methyl for up to 78 weeks					
Parameter/treatment interval	Dietary concentration (ppm)				
	0	1	5	50	500
Males					
T. Cholesterol (mg/dL), 26 weeks	114 ± 31^a	115 ± 20	166 ± 174	103 ± 20	$159 \pm 42^*$
T. Cholesterol (mg/dL), 52 weeks	123 ± 29	122 ± 53	110 ± 43	100 ± 16	173 ± 78
T. Cholesterol (mg/dL), 78 weeks	134 ± 66	115 ± 23	113 ± 58	94 ± 29	128 ± 48
Alk. Phosp. (U/L), 78 weeks	113 ± 60	89 ± 33	261 ± 342	94 ± 37	169 ± 125
Females					
T. Cholesterol (mg/dL), 26 weeks	85 ± 23	85 ± 20	69 ± 15	95 ± 25	$123 \pm 32^*$
T. Cholesterol (mg/dL), 52 weeks	75 ± 25	87 ± 11	98 ± 21	85 ± 30	$134 \pm 28^{**}$
T. Cholesterol (mg/dL), 78 weeks	76 ± 22	80 ± 14	99 ± 39	108 ± 42	$116 \pm 36^{**}$
Alk. Phosp. (U/L), 78 weeks	189 ± 91	125 ± 35	$112 \pm 76^{**}$	$106 \pm 37^*$	$100 \pm 34^*$

Data taken from Table 19, pp. 81-83 and Table 20, pp. 84-86, MRID 44680602.

^aMean \pm standard deviation

* $p < 0.05$, ** $p < 0.01$, Significantly different from control.

3. Cholinesterase activity

Cholinesterase activity in plasma, red blood cells, and brain is summarized in Table 5. Cholinesterase activity in plasma was inhibited by 94-97% ($p < 0.01$ compared with controls) at all time points in 500-ppm group male and female mice, by 55-70% ($p < 0.01$ or < 0.05 compared with controls) in 50-ppm group male mice, and by 63-75% ($p < 0.01$ or < 0.05 compared with controls) in 50-ppm group female mice. Red blood cell cholinesterase activity was statistically significantly ($p < 0.01$) inhibited at all time points by 93-96% in 500-ppm group males, by 47-52% in 50-ppm group males, by 88-93% in 500-ppm group females, and by 31-33% in 50-ppm group females. Brain cholinesterase activity was statistically significantly inhibited at all time points in both male (53-64% compared with control) and female mice (45-50%) at 500 ppm. However, at 50 ppm, brain cholinesterase activity was significantly inhibited only at week 78 in males (14% compared with controls) and week 52 in females (25%). Cholinesterase activity was not statistically inhibited in either male or female mice receiving 1 or 5 ppm of the test compound.

TABLE 5. Cholinesterase activity (units/mL) in various tissues from male and female mice fed Chlorpyrifos methyl for up to 78 weeks					
Source/treatment interval	Dietary concentration (ppm)				
	0	1	5	50	500
Males					
Plasma/26 weeks	4.67 ± 1.42*	4.12 ± 0.96	4.19 ± 1.57	1.41 ± 0.51* (60) ^b	0.26 ± 0.07** (94)
Plasma/52 weeks	6.24 ± 2.30	8.29 ± 4.73	4.45 ± 1.02	2.78 ± 0.93* (55)	0.33 ± 0.12** (95)
Plasma/78 weeks	9.11 ± 4.23	7.22 ± 1.80	6.12 ± 1.67	3.10 ± 2.39** (66)	0.55 ± 0.57** (94)
Red blood cells/26 weeks	0.17 ± 0.02	0.18 ± 0.02	0.16 ± 0.02	0.09 ± 0.01** (47)	0.01 ± 0.00** (94)
Red blood cells/52 weeks	0.23 ± 0.02	0.23 ± 0.05	0.22 ± 0.04	0.11 ± 0.03** (52)	0.01 ± 0.01** (96)
Red blood cells/78 weeks	0.15 ± 0.03	0.16 ± 0.03	0.16 ± 0.04	0.08 ± 0.02* (47)	0.01 ± 0.01** (93)
Brain/26 weeks	0.32 ± 0.06	0.33 ± 0.02	0.35 ± 0.03	0.32 ± 0.04 (6)	0.15 ± 0.04** (53)
Brain/52 weeks	0.60 ± 0.09	0.61 ± 0.08	0.62 ± 0.06	0.54 ± 0.05 (10)	0.27 ± 0.05** (55)
Brain/78 weeks	0.36 ± 0.04	0.34 ± 0.02	0.34 ± 0.04	0.31 ± 0.04* (14)	0.13 ± 0.03** (64)
Females					
Plasma/26 weeks	9.29 ± 1.95	10.76 ± 1.65	8.06 ± 1.44	2.50 ± 0.47† (73)	0.34 ± 0.13** (96)
Plasma/52 weeks	7.60 ± 1.73	7.88 ± 2.75	6.45 ± 1.25	2.83 ± 0.93** (63)	0.24 ± 0.05** (97)
Plasma/78 weeks	7.76 ± 2.17	6.91 ± 1.10	6.91 ± 1.52	1.92 ± 0.47** (75)	0.29 ± 0.13** (96)
Red blood cells/26 weeks	0.09 ± 0.01	0.09 ± 0.01	0.08 ± 0.02	0.06 ± 0.01** (33)	0.01 ± 0.00** (89)
Red blood cells/52 weeks	0.15 ± 0.03	0.12 ± 0.02	0.13 ± 0.02	0.10 ± 0.02** (33)	0.01 ± 0.00** (93)
Red blood cells/78 weeks	0.16 ± 0.02	0.15 ± 0.03	0.14 ± 0.03	0.11 ± 0.02** (31)	0.02 ± 0.01** (88)
Brain/26 weeks	0.20 ± 0.01	0.20 ± 0.02	0.20 ± 0.02	0.18 ± 0.02 (10)	0.11 ± 0.02** (45)
Brain/52 weeks	0.36 ± 0.04	0.32 ± 0.06	0.30 ± 0.05	0.27 ± 0.09** (25)	0.18 ± 0.05** (50)
Brain/78 weeks	0.40 ± 0.03	0.39 ± 0.04	0.38 ± 0.04	0.38 ± 0.03 (5)	0.20 ± 0.04** (50)

Data taken from Table 21, pp. 87-89 and Table 22, pp. 90-92, MRID 44680602.

*Mean ± standard deviation

†Percent inhibition compared with the control group.

* $p < 0.05$. ** $p < 0.01$, Significantly different from control; † $p < 0.05$ calculated by the reviewer.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

There were no treatment-related differences in organ weights. A statistically significant difference from the control group was noted in group mean relative kidney weight (to body weight) of male mice in the 26-week interim group at 50 ppm, but the change was not dose related and the relative kidney weights were comparable to the control at the other time periods.

2. Gross pathology

There were no statistically significant, dose-related macroscopic findings at any time point in the study. A slightly increased incidence of pale liver was seen in high-dose males killed at the 52 weeks and those surviving to study termination (controls, 1/20 and 1/32; 500 ppm, 3/24 and 6/38, respectively) and in high-dose females killed at 52 weeks (control, 0/23; 500 ppm, 3/24). However, the increased incidences were not statistically significant and were not seen in females surviving to study termination or male or females that died or were killed at unscheduled times.

3. Microscopic pathology

a. Non-neoplastic

Selected microscopic findings are summarized in Table 6. Notable lesions occurred in the liver, kidney, and adrenal glands of male mice and in the liver of female mice. Fatty change in centrilobular hepatocytes occurred in 75% ($p<0.01$) of 500-ppm group male mice killed at 52 weeks (compared with 25% of controls) and 40% ($p<0.01$) of the main study male mice compared with 18% of controls. In females, fatty change in centrilobular hepatocytes occurred in 71% ($p<0.01$) of the animals killed at 52 weeks compared with only 4% of controls and in 40% ($p<0.01$) of main study female mice compared with only 6% of controls. Diffuse hepatocellular fatty change occurred in 15% ($p<0.05$) of 50-ppm group female mice in the main study compared with 2% of controls; the incidence at 5 ppm (9%, $p=0.07$) and 500 ppm (10%, $p=0.10$) were only marginally significant. The hepatocytes with fatty changes appeared vacuolated and contained neutral fat identified by Oil Red O stain. Tubular atrophy of the kidney was a common lesion that occurred in 34% of male controls in the main study; the incidence was 60% ($p<0.01$) in males at 500 ppm. Tubular atrophy of the kidney occurred in 49% of 50-ppm group main study male mice; this incidence was marginally increased ($p=0.08$) compared with controls. Swelling of adrenal cortical cells was observed in 42% ($p<0.01$) of 500-ppm group male mice compared with none of the 56 controls. This finding also occurred in 25% ($p<0.01$) of 500-ppm group males killed at 52 weeks but in none of the 20 controls. No treatment-related

microscopic findings were noted for male or female mice fed the 1- or 5-ppm diets.

6. Nonneoplastic histopathologic findings in male and female mice fed Chlorpyrifos methyl for up to 78 weeks					
Organ or tissue / lesion	Dietary concentration (ppm)				
	0	1	5	50	500
Males					
Liver, centrilobular hepatocellular fatty change					
52-week interim sacrifice groups	5/20 ^a	4/21	5/21	6/21	18/24 ^{**}
main study groups	10/56	17/55	12/55	12/55	21/52 ^{**}
Kidney, tubular atrophy					
52-week interim sacrifice groups	6/20	9/21	4/21	9/21	9/24
main study groups	19/56	26/55	22/55	27/55	31/52 ^{**}
Adrenal glands, cortical cell swelling					
52-week interim sacrifice groups	0/20	0/21	0/21	0/21	6/24 [*]
main study groups	0/56	0/55	0/54	0/55	22/52 ^{**}
Females					
Liver, centrilobular hepatocellular fatty change					
52-week interim sacrifice groups	1/23	2/21	0/20	3/23	17/24 ^{**}
main study groups	3/53	4/54	2/56	6/53	21/52 ^{**}
Liver, diffuse hepatocellular fatty change					
52-week interim sacrifice groups	1/23	5/21	4/20	1/23	3/24
main study groups	1/53	3/54	5/56	8/53 [*]	5/52

Data taken from Table 29, pp. 147-163, and Table 30, pp. 164-179, MRID 44680602

^aNo. mice with lesion/no. mice for which the site was examined.

*p < 0.05, **p < 0.01, Statistically significantly different from controls.

b. Neoplastic

No significant treatment-related increases in neoplasms were found in the study. A summary of the total number of animals with neoplasms seen is given in Table 7. No statistical increase was observed in the total number of animals with either benign or malignant neoplasms.

TABLE 7. Neoplastic findings in male and female mice fed Chlorpyrifos methyl for up to 78 weeks					
Organ or tissue / neoplasm	Dietary concentration (ppm)				
	0	1	5	50	500
Males					
No. animals per group					
52-week interim sacrifice groups	20	21	21	21	24
main study groups	56	55	55	55	52
Total no. mice with benign neoplasm(s)					
52-week interim sacrifice groups	8	10	5	9	9
main study groups	24	26	33	32	30
Total no. mice with malignant neoplasm(s)					
52-week interim sacrifice groups	1	2	5	3	2
main study groups	23	17	26	18	22
Total no. mice with neoplasm(s)					
52-week interim sacrifice groups	8	12	9	11	10
main study groups	37	37	46	42	42
Females					
No. animals per group					
52-week interim sacrifice groups	23	21	20	23	24
main study groups	53	55	56	53	52
Total no. mice with benign neoplasm(s)					
52-week interim sacrifice groups	1	2	4	3	3
main study groups	20	13	23	20	21
Total no. mice with malignant neoplasm(s)					
52-week interim sacrifice groups	1	2	1	5	2
main study groups	20	21	20	22	18
Total no. mice with neoplasm(s)					
52-week interim sacrifice groups	2	3	5	7	5
main study groups	36	31	37	33	33

Data taken from Table 27, pp. 132-138, and Table 28, pp. 139-146, MRID 44680602.

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSION

The investigators concluded that the administration of up to 500 ppm Chlorpyrifos methyl in the diet of mice for up to 78 months resulted in no significant increases in the incidences of any type of neoplastic lesions compared to control animals.

Treatment at 500 ppm resulted in slightly lower body weights in males throughout the study and in females during the first year of treatment. High-dose males had decreased

food consumption and food efficiency early in the study and both sexes had decreased water consumption compared to the controls. Clinical chemistry showed increased cholesterol values in males at weeks 26 and 52 and in females at all intervals at 500 ppm compared to the controls. Decreased cholinesterase activity was seen at all intervals in plasma, red blood cells, and brain in both sexes at 50 and 500 ppm compared to the control animals. Histopathology examination revealed increased incidences of centrilobular hepatocellular fatty change in both sexes at 500 ppm compared to the controls. Increased incidences of adrenal cortical cell swelling, renal tubular atrophy, and renal cortical cyst(s) were seen in males at 500 ppm; the overall incidence of tubular atrophy was also increased in males at 50 ppm compared to the controls. No dose-related abnormalities were seen at 5 ppm or 1 ppm compared to the control groups.

B. REVIEWER'S DISCUSSION

There were no treatment-related changes in clinical signs or survival seen in the study. The mean body weight of high-dose males was slightly lower than the control throughout the study, but was only sporadically statistically significant. The body weight gain of high-dose males was decreased by about 12% at 52 weeks and by about 17% at 78 weeks compared to the control group. Food consumption was also slightly decreased in high-dose males during the first 12 weeks of the study, and the overall food efficiency of high-dose males was lower than the control group although not significantly, and is indicative of a toxic effect of treatment. The overall body weight, weight gain, food intake, and food efficiency in high-dose females were comparable to the control group. Water consumption was decreased in both sexes at 500 ppm. The decrease in males was only 7% compared with controls, which is not considered biologically significant. The overall decrease in females was 14% but it did not achieve statistical significance.

No significant treatment-related changes were seen in hematology parameters. Blood clinical chemistry revealed increased total blood cholesterol in high-dose males at 26 weeks (increased by 39%, $p < 0.05$) and in high-dose females at all time points compared to the controls (increased by 45-79%, $p < 0.05$ or 0.01). The 41% increase in total cholesterol observed in 500-ppm group males at 52 weeks did not achieve statistical significance. The increased cholesterol may be associated with the microscopic fatty changes seen in the livers of high-dose males and females and is probably treatment-related. In addition, the animals were not fasted before blood was taken, which may have affected the cholesterol level. Alkaline phosphatase activity was decreased in all treated females, but there was no clear dose effect and the toxicological significance of this observation is unknown. Urinalysis data showed inconsistent effects particularly for male mice. The data showed statistically significant changes in some urinalysis parameters at various time points (specific gravity, pH, and protein content). However, some of the changes were inconsistent with histopathologic findings suggesting that the changes may have been an artifact of the collection procedure. For example, the urine protein content in male controls was severely elevated compared with 500-ppm group, yet the treated group had a statistically significant increase in the incidence of renal tubular atrophy. The

decreased urine protein is not considered treatment-related. Other urinalysis changes (specific gravity) were very small and clearly biologically insignificant.

Increased incidences of pale liver seen upon gross necropsy in high-dose males at 52 and 78 weeks and in high-dose females at 52 weeks, though not statistically significant, were probably associated with the significantly increased incidence of microscopic fatty changes in centrilobular hepatocytes of high-dose males and females at 52 and 78 weeks. The centrilobular hepatocellular fatty changes are considered to be treatment-related. The incidence of diffuse hepatocellular fatty change was significantly increased in main study females at the 50-ppm dietary level and marginally increased at 5- and 500-ppm dietary levels. A marginal increase in the incidence of diffuse fatty changes was also observed at the 1-ppm dietary level in female mice sacrificed at 52 weeks. No clear-dose response relationship was observed for either the 52-week interim sacrifice or main study animals, suggesting that the increased incidence of this lesion is not treatment related.

A statistically significant increased incidence of kidney tubular atrophy was seen in 500-ppm group males in the main study and a marginally significant increase was seen for the 50-ppm group. Kidney tubular atrophy occurred in 34% of male controls; the incidence at 50 ppm was 49% ($p=0.08$). Because the incidence did not show a clear statistically significant increase for a high background lesion, kidney tubular atrophy at 50 ppm is not considered treatment-related. The incidence of kidney cortical cysts was also increased in high-dose males surviving to study termination, but the incidence was not statistically increased when all main study animals were combined. The incidence of adrenal cortical swelling was significantly increased in high-dose males assigned to the 52-week interim sacrifice and main study groups. The incidence was also higher at 52 weeks (71%) than in the main study group (40%). This lesion was not found in any of the 76 male mice assigned to the control groups.

The lowest-observed-adverse-effect-level (LOAEL) for systemic toxicity for this study is 500 ppm for both sexes (44.0 mg/kg/day for males and 41.5 mg/kg/day for females) based on histopathologic lesions in the liver, kidney, and adrenal glands of male mice and in the liver of female mice. The no-observed-adverse-effect-level (NOAEL) is 50 ppm (4.40 mg/kg/day for males and 3.94 mg/kg/day for females).

Cholinesterase activity from plasma and red blood cells was moderately inhibited at 50 ppm by 47-70% in male mice and by 31-75% in female mice and severely inhibited at 500 ppm by 93-96% in male mice and by 88-97% in female mice at all time points (26, 52, and 78 weeks). Brain cholinesterase activity was significantly decreased by 53-64% in 500-ppm group male mice and by 45-50% in 500-ppm group female mice at all time points. At 50 ppm, brain cholinesterase activity in males, was statistically significantly decreased (14%) only at week 78 and in female mice (25%) only at week 52.

Therefore, the LOAEL for inhibition of cholinesterase activity is 50 ppm for both sexes (4.40 mg/kg/day for males and 3.94 mg/kg/day for females). The NOAEL is 5 ppm (0.418 mg/kg/day for males and 0.403 mg/kg/day for females).

Treatment of specific pathogen free ICR Crj:CD-1® mice for up to 78 weeks did not result in a significant increase in the incidence of neoplastic lesions in this study. Dosing was adequate as evidenced by inhibition of cholinesterase activity at 50 ppm in both sexes and treatment-related microscopic lesion at 500 ppm in both sexes.

C. STUDY DEFICIENCIES

The homogeneity of the dietary mixtures was tested in the 28-day dose range-finding study in which the chlorpyrifos methyl was apparently not dissolved in acetone prior to mixing with the diet as it was in this study. This could have resulted in different values for homogeneity.

APPENDIX I

CHLORPYRIFOS-METHYL: 28-DAY ORAL TOXICITY STUDY IN MICE

SPECIAL DOSE SELECTION STUDY

MRID NO: 44668202

Study type: Supplementary range-finding study for the oncogenicity (83-6) study

Test material: Chlorpyrifos-methyl (91.8% a.i.)

Laboratory study ID: GHF-R-80

Testing facility: The Institute of Environmental Toxicology, Suzuki-cho 2-772, Kodaira-shi,
Tokyo 187, Japan

Study title: Chlorpyrifos-methyl: 28-day oral toxicity study in mice

Study author(s): Yoshida, A., et al.

Report date: October 2, 1985

METHODS

Test animals: Specific pathogen free ICR Crj:CD-1[®] obtained from Charles River Japan, Inc.,
Shimofurusawa, Atsugi-shi, Kanagawa

Group size: 12 males and 12 females per group

Test concentrations: 0, 1, 5, 10, 1000, or 10,000 ppm

Chemical intake: males: 0, 0.125, 0.651, 1.27, 122, or 523 mg/kg/day; females: 0, 0.141, 0.745,
1.45, 139, or 318 mg/kg/day

Duration of treatment: 28 days

Observations made: Clinical signs, daily; body weight, food consumption, chemical intake,
food efficiency and water consumption, weekly; urinalysis, week 4;
hematology, blood chemistry, cholinesterase in plasma, red blood cells,
and brain, and full autopsy and histopathology at 28 weeks. Cholinesterase
measurements were made on the first 6 animals in each group; hematology
and blood chemistry measurements were made on the remaining animals.
Clinical tests were not performed on animals in the 10,000 ppm groups.

RESULTS

Clinical signs:

Marked weight loss, small body size, and emaciation were noted at 10,000 ppm in both
sexes. No treatment-related signs were seen at the other doses.

Mortality:

All animals of both sexes fed the 10,000-ppm diet died or were killed *in extremis* by the 9th
day of treatment. No deaths occurred during the study at the lower doses.

Body weight:

The animals in the 10,000 ppm group were about half the weight of the control animals at
the time of their death. The mean body weight of males at 1000 ppm was about 93% ($p < 0.05$) of the control group and females were about 96% (NS) of the control group at
treatment termination. There were no other treatment-related body weight differences noted.

Food and water consumption:

Marked decreased food consumption was seen in both sexes at 10,000 ppm during the first
week of treatment (about 20% of control groups). Mean food consumption in males at 1000

ppm was about 94% of the control and in females was about 93% of the control group for the entire treatment period. No other differences in food consumption were seen. Water consumption for males and females after 1 week of treatment at 10,000 ppm was 36% and 43% of the controls, respectively. The total mean water consumption for the 1000-ppm group was 93% and 82% of the control values for males and females, respectively. There were no other differences that could be related to treatment.

Food efficiency:

The food efficiency values were negative for both sexes at 10,000 ppm due to the marked weight loss. Decreased food efficiency was noted in both males and females (60% and 53% of controls, respectively) after 1 week of treatment at 1000 ppm and the overall mean food efficiency was decreased in males (76% of controls) and females (84% of controls) after 4 weeks of treatment.

Hematology and clinical chemistry:

No significant treatment-related hematologic changes were noted compared to the controls. Clinical chemistry: The total mean blood cholesterol was increased 160% (NS) and 155% ($p < 0.01$) of the controls in males and females, respectively after 4 weeks of treatment at 1000 ppm. The glutamic pyruvic transaminase (GPT) activity was also increased 131% (NS) of the control in males and 150% ($p < 0.05$) of the control in females after 4 weeks at 1000 ppm.

Cholinesterase activity:

The cholinesterase activity in plasma, red blood cells, and brain after 4 weeks of treatment with chlorpyrifos methyl is given in Table A1. The cholinesterase activity from all sources was markedly decreased in both sexes tested at 1000 ppm and significantly decreased in plasma of males at 10 ppm and in red blood cells and plasma of females at 5 and 10 ppm.

TABLE A1. Cholinesterase activity (units/ml) in mice after 4 weeks of treatment with Chlorpyrifos methyl					
Source	Dietary concentration (ppm)				
	0	1	5	10	1000
Males					
Plasma	3.59 ± 1.22^a	3.54 ± 0.58	2.66 ± 0.71	$1.69 \pm 0.44^*$	$0.15 \pm 0.02^{***}$
Red blood cells	0.15 ± 0.04	0.16 ± 0.03	0.15 ± 0.01	$0.10 \pm 0.01^*$	$0.00 \pm 0.00^{***}$
Brain	0.28 ± 0.02	0.25 ± 0.02	0.24 ± 0.04	0.25 ± 0.04	$0.08 \pm 0.01^{***}$
Females					
Plasma	5.40 ± 0.60^a	4.85 ± 0.85	$4.46 \pm 0.78^*$	$4.14 \pm 0.59^{**}$	$0.14 \pm 0.03^{***}$
Red blood cells	0.13 ± 0.02	0.13 ± 0.02	0.12 ± 0.01	0.11 ± 0.02	$0.00 \pm 0.00^{***}$
Brain	0.23 ± 0.02	0.22 ± 0.03	0.24 ± 0.03	0.24 ± 0.02	$0.06 \pm 0.01^{***}$

Data taken from Table 21-2, p. 47 and Table 22-2, p. 49, MRID 44668202

^amean \pm standard deviation

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, significantly different from the control.

Organ weights:

013399

The absolute and relative (to body weight) mean liver weights were increased in males at 1000 ppm (120% and 127% of control, respectively, $p < 0.01$). The relative liver weight was increased in females at 1000 ppm (109% of control, $p < 0.05$); however, the difference partly reflects the slightly decreased female terminal body weight at 1000 ppm compared to the control. The absolute mean adrenal weight was increased to 122% ($p < 0.05$) of the controls in males at 1000 ppm and the relative adrenal weight (to body weight) was increased to 127% ($p < 0.01$) of the controls.

Histopathology:

Liver hepatocellular atrophy, splenic atrophy, and pancreatic acinar cell atrophy were seen in all animals at 10,000 ppm. Cytoplasmic swelling of zona fasciculata cells in the adrenal cortex was seen in all males, in only one female at 1000 ppm, and in none of the controls of either sex.

Conclusions:

The animals in the 10,000 ppm groups consumed less food and very little water during the study and either died or were killed *in extremis* by the 9th treatment day. Starvation likely played a major roll in causing their deaths. The LOAEL for systemic effects was 10,000 ppm (523 mg/kg/day for males and 318 mg/kg/day for females) for both sexes based on mortality and associated effects; the NOAEL was 1000 ppm (122 mg/kg/day for males and 139 mg/kg/day for females).

The LOAEL for inhibition of cholinesterase activity was males was 10 ppm (1.27 mg/kg/day) in males based on decreased cholinesterase activity in plasma and red blood cells, and was 5 ppm (0.745 mg/kg/day) in females based on decreased cholinesterase activity in plasma. The NOAEL for inhibition of cholinesterase was 5 ppm for males (0.651 mg/kg/day) and 1 ppm for females (0.141 mg/kg/day). The 1000 ppm concentration resulted in markedly decreased cholinesterase activity in plasma, red blood cells, and brain in both sexes compared to the control groups (decreased by 96- 97%, 100%, and 71-74%, respectively, $p < 0.001$).

[CHORPYRIFOS-METHYL/1992]

Developmental Study OPPTS 870.3700 (\$83-3a)

EPA Reviewer: Roger Hawks, Ph.D. *Roger Hawks*

Date 2/8/94

RCAB (7509C)

EPA Secondary Reviewer: John Doherty, Ph.D. *John Doherty*

Date 2/22/95

RCAB (7509C)

DATA EVALUATION RECORD

013399

STUDY TYPE: Prenatal Developmental Study - Rat ; OPPTS 870.3700
[\$83-3a]

DP BARCODE: D250872

SUBMISSION CODE: S551248

P.C. CODE: 059102

TOX. CHEM. NO.:

TEST MATERIAL : Chlorpyrifos-methyl (96.9%)

SYNONYMS: Reldan, O'-O-dimethyl O-(3,5,6,trichloro-2-pyridyl)
phosphorthioate

CITATION: Bryson, A.M. (1992) A study of the effect of
technical Reldan on the pregnancy of the rat.
Huntingdon Research Centre Ltd.. Laboratory study
ID: GHE-413. Feb. 27, 1992 MRID 44680603.
Unpublished

SPONSOR: Dow Chemical Company

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID 44680603) chlorpyrifos-methyl, 96.9% a.i., was administered to female Sprague-Dawley rats by gavage, 30/dose/group, at dose levels of 0, 1.0, 12.5, or 50 mg/kg/day from days 6 through 15 of gestation. The control group contained 25 pregnant animals, the 1.0 mg/kg group (LDT) contained 23, the 12.5 mg/kg group (MDT) 24, and the 50 mg/kg group (HDT) contained 26.

Post dosing salivation in 10% of the HDT group was the only clinical sign of treatment. There were no treatment-related effects on maternal body weight, food consumption, or mortality. Cholinesterase levels were measured on gestation day 20 - 5 days after dosing ended. Red blood cell AChE was inhibited in the MDT (33%, $p < 0.05$) and HDT (47%) dose groups. Plasma ChE was inhibited 8%, 8% and 13% for the LDT, MDT and HDT with only the HDT being statistically significant ($p < 0.05$). Brain AChE was inhibited in the HDT (11%, $p < 0.05$).

The maternal LOAEL is 12.5 mg/kg/day, based on decreased RBC cholinesterase levels. The maternal NOAEL is 1.0 mg/kg/day.

There was no indication of developmental toxicity at any dose in this study. There were no increases in fetal/embryonic deaths or resorptions. Sex ratios were not altered. There were no increases

in either visceral or skeletal malformations or anomalies.

A developmental LOAEL was not determined. The developmental NOAEL is greater than 50 mg/kg/day, based on the lack of developmental toxicity at 50 mg/kg/day.

The developmental toxicity study in the rat is classified **Acceptable-Guideline** and does satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3a) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS1. Test Material: Chlorpyrifos-methyl

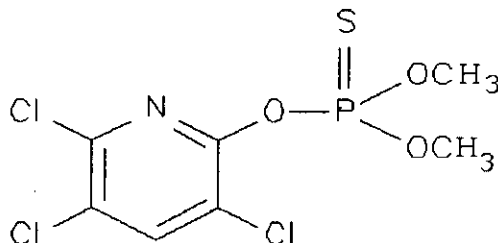
Description: amber waxy solid

Lot/Batch #: EK 900512002/RMM 1710

Purity: 96.9% a.i.

CAS #:5598-13-0

Structure-

2. Vehicle: Corn oil

Description: None given

Lot/Batch #: not given

Purity: not given

3. Test animals: Species: Rat

Strain: Cr1: CD® (SD) BR VAF/Plus Strain

Age at mating: 8 to 10 wks

Weight at mating was not noted in the study report.

Weight of females on day 2 (when females were randomly assigned to groups) of pregnancy was from 160 to 264 grams

Source: Charles River U.K.

Housing: Five animals per cage in cages with wire mesh front, floor and top and solid sides and back.

Diet: Biosure Laboratory animal Diet No. 1 ad libitumWater: Tap Water ad libitum

Environmental conditions:

Temperature: 21 +/- 3° C

Humidity: 52 +/- 6%

Air changes: not reported in the study report

Photoperiod: 12 hrs dark/12 hrs light

Acclimation period (P): animals were received from the supplier pregnant, and there was thus no acclimation period.

B. PROCEDURES AND STUDY DESIGN

1. In life dates - start: animals arrived 4/10/91 and dosing started 4/15/91 end: Sacrifice was on 5/1/91.
2. Mating: Mating was performed at the breeders by pairing females with males. Mating was considered to have occurred when sperm appeared in the vaginal smear or a vaginal plug was observed.
3. Animal Assignment: Animals were assigned to dose groups as indicated in Table 1. Assignment was random.

TABLE 1: Animal Assignment

Test Group	Dose (mg/kg/day)	# of Females Mated	# of Pregnant Females
Control	0	30	25
Low (LDT)	1.0	30	23
Mid (MDT)	12.5	30	24
High (HDT)	50.0	30	26

4. Dose selection rationale:
Doses in this study were selected based on the results of preliminary toxicity study done with pregnant rats (Huntingdon Research Center Laboratory ID # DWC/606). Doses in this study were 0, 12.5, 50 and 200 mg/kg/day. In this study cholinesterase inhibition was measured shortly after the end of dosing on day 15, rather than 5 days after dosing on day 20, as was done in the current study. Inhibition of brain cholinesterase activity was 10, 24 and 55% at the 12.5, 50 and 200 mg/kg/day dose levels. The inhibitions seen in plasma and RBC cholinesterase were higher than the brain inhibitions. Based on those decreases it was decided that 50 mg/kg was an adequate high dose.
5. Dosage preparation and analysis

Test substance formulations for the 50 mg/kg dose was prepared by mixing appropriate amounts of test substance with the required amount of corn oil to prepare a 2.5% w/v solution. The 2 lower doses were prepared by serial dilution. All doses were prepared fresh daily. Analysis Stability of the test substance in corn oil was evaluated for a period of 24 hours (storage conditions not given). Concentration was evaluated at the second day of dosing.

Results -

Stability Analysis:

Nominal Concentration	Storage time(hr)	Analysis I in mg/ml	Analysis II in mg/ml	Mean in mg/ml
0.5 mg/kg	0	0.508	0.497	0.502
	4	0.493	0.511	0.502
	24	0.510	0.509	0.510
1 mg/ml	0	0.989	0.994	0.992
	4	0.980	0.99	0.989
	24	1.01	1.09	1.01
200 mg/ml	0	201	199	200
	4	202	203	203
	24	203	205	204

Concentration Analysis:

Group	Nominal Concentration	Analysis I	Analysis II	Mean Measured concentration
control	0	none detected	none detected	none detected
LDT	0.5	0.513	0.525	0.519
MDT	6.25	6.39	5.93	6.16
HDT	25	25.5	25.3	25.4

Stability data from page 91, current study and concentration data from page 90, current study.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. Dosage administration: All doses were administered once daily by gastric intubation on gestation days 6 through 15, in a volume of 0.2 ml/kg of body weight/day. Dosing was based initially on the body weight on gestation day 6 and then adjusted at days 8, 10, and 12.

C. OBSERVATIONS

1. Maternal Observations and Evaluations - The animals were checked for mortality or clinical signs daily. Body weight and food consumption data were recorded on gestation days 2, 3, 6, 8, 10, 12, 14, 16, 18, and 20. Dams were sacrificed on day 20 of gestation. Examinations at sacrifice consisted of gross necropsies,

and examination of the ovaries and uteri for corpea lutea, live young, and embryofetal deaths. The brain was also removed and the left half was weighed and prepared for cholinesterase determination.

2. Fetal Evaluations - The fetuses were weighed and examined for external fetal abnormalities. Half the fetuses were then preserved in Bouin's solution for examination of the viscera and half were eviscerated, cleared and stained with alizarin red for skeletal evaluation by Dawson's technique.
3. Cholinesterase Measurements - Blood was drawn from the orbital sinus of each animal immediately before sacrifice on day 20. This blood was collected into tubes with heparin and analyzed by spectrophotometry for plasma and erythrocyte (RBC) cholinesterase activity. At necropsy the brain was removed, the left half weighed and prepared for brain cholinesterase activity measurements.

D. DATA ANALYSIS

1. Statistical analyses: Mean litter data values were analyzed by the Kruskal-Wallis test. Intergroup comparisons were made by the non-parametric equivalent of the t-test together with the Jonckheere test for an ordered series of treatments. Cholinesterase levels were examined with a parametric analysis of variance followed by William's test.

Reference for Kruskal-Wallis and jonckheere tests: Non-Parametric Statistical Methods. Hollander, M. and Wolfe, D.A., 1973. J. Wiley and Sons, N.Y.

2. Indices: The following indices were calculated from cesarean section records of animals in the study:

Pre-implantation loss -

$$\frac{\# \text{ of corpea lutea} - \# \text{ of implantations}}{\# \text{ of corpea lutea}} \times 100$$

Post-implantation loss -

$$\frac{\# \text{ of implantations} - \# \text{ of live young}}{\# \text{ of implantations}} \times 100$$

3. Historical control data: Historical control data for malformations were provided.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: There were no unscheduled deaths during the study. The only compound related clinical sign reported was post-dosing salivation in 3 of the HDT animals (day 10 for one animal, day 12 for the second and day 13 for the 3rd).
2. Body Weight - Body weight data are summarized in Tables 2 and 3. Compound exposure had no effect on body weights or body weight gains.

TABLE 2: Mean maternal body weight in grams at selected timepoints (pregnant animals only).

	Day 2	Day 8	Day 14	Day 16	Day 20
Control	217.8	266.1	312.8	332.6	393.8
LDT	220	267.3	314.7	334.7	397.4
MDT	220	265.3	310.9	329.7	387.9
HDT	216.5	265.9	313	333.1	393.7

Data from page 29, current study

TABLE 3: Maternal Body Weight Gain in grams (pregnant animals only).

Interval	Dose in mg/kg/day (# of Dams)			
	Control (25)	LDT (23)	MDT (24)	HDT (26)
Pretreatment: Days 2 -6	34.6	35.4	32.2	34.9
Treatment: Days 6-16	80.2	79.3	77.6	81.7
Posttreatment: Days 16-20	61.2	62.7	58.2	60.5

Data extracted from page 29 current study

3. Food Consumption - Exposure to the test article did not affect food consumption.

4. Gross Pathology - Only 5 dams had necropsy findings. Four of these dams (one in each dose group) had scabbing of the skin and the fifth (in the MDT) had an additional lobe on the liver. These findings are not related to compound exposure.
5. Plasma, RBC and brain cholinesterase levels
 Plasma, RBC and brain cholinesterase levels were statistically significantly reduced at the HDT compared to controls. RBC cholinesterase levels were statistically significantly reduced at the MDT while plasma and brain levels were also reduced, but not statistically significantly. At the LDT, plasma cholinesterase levels were reduced, but not statistically significantly. RBC and brain cholinesterase levels were slightly higher than controls at the LDT.

Table 4: Mean group cholinesterase levels

	Plasma ml/min	RBC ml/min	Brain g/min
Control	\bar{x} = 1.6 SD= 0.226	\bar{x} = 1.59 SD=0.53	\bar{x} = 8.01 SD= 0.893
LDT	\bar{x} = 1.47 SD=0.267	\bar{x} = 1.6 SD=0.390	\bar{x} = 8.67 SD=0.782
MDT	\bar{x} = 1.48 SD=0.172	\bar{x} = 1.06* SD=0.387	\bar{x} = 7.93 SD=0.772
HDT	\bar{x} = 1.4* SD=0.228	\bar{x} = 0.85* SD=0.346	\bar{x} = 7.09* SD=0.725

* $p < 0.01$ compared to control

Values taken from pages 30, and 48 to 51, current study

6. Cesarean Section Data - There were no alterations in cesarean section data in dosed groups compared to controls. Table 5 displays cesarean section parameters.

TABLE 5: Cesarean Section Observations

Observation	Dose (mg/kg/day)			
	0	LDT	MDT	HDT
# Animals Assigned (Mated)	30	30	30	30
# Animals Pregnant Pregnancy Rate (%)	25 (83%)	23 (77%)	24 (80%)	26 (87%)
# Nonpregnant	4	7	5	4
Maternal Wastage	1*	0	1*	0
# Died	1	0	1	0
# Died Pregnant	1		1	
# Died Nonpregnant	0		0	
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Total # Corpora Lutea Corpora Lutea/Dam	330 13.2	310 13.5	334 13.9	372 14.3
Total # Implantations Implantations/Dam	298 11.9	283 12.3	295 12.3	330 12.7
Total # Litters	25	23	24	26
Total # Live Fetuses Live Fetuses/Dam	278 11.1	271 11.8	284 11.8	315 12.1
Total # Dead Fetuses Dead Fetuses/Dam	0	0	0	0
Total # Resorptions	20	12	12	16
Early	19	11	9	15
Late	1	1	3	1
Resorptions/Dam	0.8	0.5	0.5	0.6
Early	0.8	0.5	0.4	0.6
Late	0.04	0.04	0.1	0.04
Litters with Total Resorptions	0	0	0	0
Mean Fetal Weight, both sexes combined(g)	3.73	3.76	3.7	3.89
Sex Ratio (% Male)	43%	52.1%	46.7%	50.6%
Preimplantation Loss (%)	10.4%	10%	12.7%	10.1%
Postimplantation Loss (%)	9.4%	4.1%	3.9%	4.9%

* At sacrifice it was found that the mating was mistimed and so these animals were not included in this analysis.
Data extracted from pages 31, and 52 to 55, current study.

B. DEVELOPMENTAL TOXICITY

1. External Examination - Although the study states that all fetuses were examined externally, nowhere are the results of the external examinations presented. Table 8, on page 34, lists only malformations and does not specify whether these malformations were seen at external, visceral or skeletal examination. Results of external examinations are not listed in Appendix 7 and they are not described in the "Results" section of the study report.
2. Visceral Examination - There were few findings at visceral examination. None of these findings were compound related. The most common findings are displayed in Table 6a.
3. Skeletal Examination - There were few findings at skeletal examination. None of these findings were compound related. The most common findings are displayed in Table 6b.

TABLE 6a. Visceral Examinations

Observations+	Dose (mg/kg/day)			
	0	LDT	MDT	HDT
#Fetuses(litters) examined	140 (25)	136 (22)	142 (23)	155 (26)
#Fetuses(litters) affected	11 (8)	4 (4)	11 (9)	15 (12)
Interventricular septal defect (anomaly)	4 (3)	1 (1)	3 (2)	5 (5)
Interventricular septal defect (malformation)	0	1 (1)	0	2 (2)

Data from pages 34 and 35, current study

TABLE 6b. Skeletal Examinations

Observations	Dose (mg/kg/day)			
	0	LDT	MDT	HDT
#Fetuses(litters) examined	137 (24)	133 (23)	138 (24)	158 (26)
#Fetuses(litters) affected	16 (8)	14 (12)	14 (11)	8 (8)
Unossified sacrocaudal vertebral archs	12 (6)	4 (3)	6 (5)	5 (5)
Distorted ribcage	0	0	4 (1)	0
Forelimbs flexures/malrotated hindlimbs	0	0	4 (1)	0

Data from pages 34 and 36, current study

III. DISCUSSION**A. INVESTIGATORS' CONCLUSIONS**

The study author concluded that the only treatment-related effect on the dams were the decreases in RBC, plasma, and brain cholinesterase seen at the HDT; the decreases in RBC cholinesterase seen at the MDT; and the post-dosing salivation seen in 10% of the HDT animals. The study author did not believe there were any obvious adverse effects of dosing on developmental parameters.

The study author considered the maternal LOAEL and NOAEL to be 12.5 and 1.0 mg/kg/day and the developmental NOAEL to be above 50 mg/kg/day.

013399

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: The reviewer agrees that the only treatment-related clinical signs of maternal toxicity are cholinesterase inhibition and post-dosing salivation in a few animals of the HDT. A statistically significant decrease (33%) was seen in RBC cholinesterase at the 12.5 mg/kg/day. Based on this effect, the maternal LOAEL is considered by the reviewer to be 12.5 mg/kg/day. The maternal NOAEL is 1.0 mg/kg/day.
2. DEVELOPMENTAL TOXICITY:
 - a. Deaths/Resorptions: There was not a dose-related increase in feto/embryonic deaths or resorptions.
 - b. Altered Growth: There were no dose-related alterations in fetal or embryonic growth.
 - c. Developmental Variations: There were no dose-related increases in developmental variations.
 - d. Malformations: There were no dose-related increases malformations.

Exposure to doses of chlorpyrifos-methyl as high as 50 mg/kg/day did not result in alterations of the measured developmental parameters. The LOAEL for developmental effects is greater than 50 mg/kg/day.

This study is considered Acceptable-Guideline, and satisfies the guideline requirement for a prenatal developmental study in the rat - OPPTS 870.3700 [\$83-3a].

- C. STUDY DEFICIENCIES: Table 2 and 3a and 3b, in the study, do not list standard deviations. It would be preferable if these were displayed. Results of external examination are not displayed and nowhere in the text can any mention of these results be found. Table 8 on page 34 is not clearly written. It is not clear from this table which malformations were seen in visceral and which were seen in skeletal examination. It is somewhat confusing that in the column labeled "Foetuses" it notes that 278, 271, 284 and 315 foetuses were examined. Some of the malformations, such as duplicated vena cava and interventricular septal defect were not likely to be seen on skeletal examination. These are malformations that were most likely seen on visceral examination with the Wilson Technique. Yet only half the foetuses were examined by this technique, not all of them as the table would imply. These deficiencies do not alter the classification of this study as acceptable-guideline.

[Chlorpyrifos-methyl, 1990]

Subchronic Oral Study (82-1 b)

EPA Reviewer: Roger Hawks, Ph.D. Roger Hawks, Date 3/15/99
RCAB (7509C)

EPA Secondary Reviewer: John Doherty, Ph.D. John Doherty, Date 3/15/99
RCAB (7509C)

013399

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity feeding- dog; 870.3151
(nonrodent) [§82-1 b)

DP BARCODE: D250872

SUBMISSION CODE: S551248

P.C. CODE: 059102

TOX. CHEM. NO.:

TEST MATERIAL (PURITY): Chlorpyrifos-methyl (95.2 +/- 0.4%)

SYNONYMS: Reldan, O'-O-dimethyl O-(3,5,6, trichloro-2-pyridyl)
phosphorothioate

CITATION: J.R. Szabo and B.L. Rachunek (1990) Chlorpyrifos-
Methyl (Reldan): 13-week dietary toxicity study in
Beagle dogs. Lake Jackson Research Center, Freeport,
Texas. Laboratory report number: TXT:K-046193-027.
April 17, 1990. MRID 44680601. Unpublished.

SPONSOR: Dow Chemical Company

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID #44680601), four groups of 4/sex beagle dogs were dosed as control, 0.1, 10 or 50 mg/kg/day with chlorpyrifos methyl (95.2%) for a period of 13 weeks. The dose level was achieved by periodically adjusting the chlorpyrifos methyl content of the diet based on the weekly food consumption and body weight data.

At 0.1 mg/kg/day **plasma ChE** was statistically significantly inhibited (11% ♂, 14% ♀) at week 6 and at termination (14% ♂ only) and approximately 50% inhibition was noted at 10 mg/kg/day. **RBC AChE** was inhibited starting at 10 mg/kg/day (28% ♂, 29% ♂ at week 6 and 25% ♂ and 20% ♀ at termination). Inhibition was only slightly higher at 50 mg/kg/day. **Brain AChE** was inhibited only at 50 mg/kg/day (66% ♂ and 70% ♀). **The LOAEL for inhibition is 10 mg/kg/day based on 50% inhibition of plasma ChE and 25% inhibition of RBC AChE. The NOAEL is 0.1 mg/kg/day.** The 11-14% inhibition of plasma ChE at 0.1 mg/kg/day is noted but not incorporated into the LOAEL.

Systemic effects were evident only at 50 mg/kg/day and included **body weight** decreases (10-18% for both sexes), decreased food consumption (10-29% ♂ and 32-33% ♀ possibly due to poor palatability of the test material). One **death** (a female, moribund sacrifice in the final days of the study) was attributed to muscle wasting and inability to rise possibly (but not definitely) related to diet refusal. A second death in the first week of the study was unexplained. Other effects considered possibly related to diet refusal included decreases in RBC, hemoglobin and PCV and increases in platelets and creatinine kinase and skeletal muscle degeneration in females.. There

were also *increases* in alkaline phosphatase (slight). There were *decreases* in albumin, BUN, total protein and cholesterol. Pathology indicated **centrilobular hypertrophy in liver** (3/4 ♂ and 4/4 ♀). **The systemic LOAEL is 50 mg/kg/day based mainly on body weight and food consumption decreases and an associated death and liver pathology as well as other effects. The systemic NOAEL is 10 mg/kg/day.**

This subchronic toxicity study is classified **Acceptable-Guideline** and *does* satisfy the guideline requirement for a subchronic oral study (82-1) in the dog.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

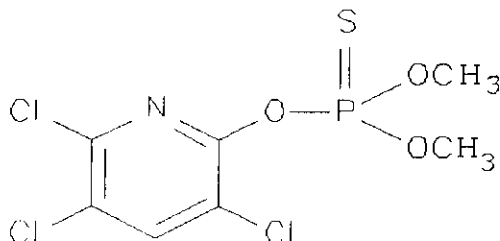
I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Chlorpyrifos-methyl (AGR-219561)

Description: granular crystal

Lot/Batch #: The lot # used in the range-finding study (MRID 44668201) described in the supplement to this review was EK840911089. The lot used in this study was not specified
Purity: 95.2 +/- 0.4% ai.



Stability of
compound: not specified
CAS #: 5598-13-0

Structure-

2. Vehicle and/or positive control: test article was dissolved in acetone, Lot/Batch number not given.

3. Test animals: Species: Dog

Strain: Beagle

Age and weight at study initiation: age- 23 weeks;

Mean wt. males - 9.736kg,

Mean wt. females - 8.645 kg.

Source: Laboratory Research Enterprises, Kalamazoo MI

Housing: segregated by sex, 2 per pen in wire mesh pens

Diet: Purina Certified Canine Diet #5007 ad libitum

Water: Municipal tap water ad libitum

Environmental conditions: Temperature: 75 +/- 5° F

Humidity: 30-70%

Air changes: not recorded

Photoperiod: 12 hour

Acclimation period: 7 weeks

B. STUDY DESIGN:

1. In life dates - start: 1/5/88 end: 4/8/88

2. Animal assignment

Animals were randomly assigned to the test groups in table 1.

TABLE 1: STUDY DESIGN

Test Group	Target Conc. in Diet in mg/kg/day	Male	Female
Control	0	4	4
Low (LDT)	0.1	4	4
Mid (MDT)	10	4	4
High (HDT)	50	4	4

3. Dose Selection

Doses were selected based on the results of a previous range-finding study (MRID 44668201). A brief summary of the results of this study are included as a supplement to this review.

4. Diet preparation and analysis

Diet was prepared weekly by mixing appropriate amounts of chlorpyrifos-methyl dissolved in acetone with Purina Certified Canine Diet #5007. How the diet mixture was stored was not recorded in the study report. Homogeneity of the test article in Purina #5007 was tested in a

previous study (AL Report DT-7-88). Stability for 28 days was tested in a previous study (Hinze, C.A. 1988a). During the study, samples of treated food were analyzed at study day 1 and 85 for concentration.

The concentration of the test article to be dissolved in acetone and added to the diet was determined based on the animals previous weeks weight and food consumption and the desired dose. For example: the targeted concentration in the food for the diet mixture prepared on study day one (to be consumed the first week of the study) was 2.51 μ g test article per gram of diet. The mean food consumption for the males and females assigned to this dose group for the previous week was 397.2 grams per animal per day and the mean body weight for males and females assigned to this dose group was 9.65 kg. Thus;

$$\frac{0.00251 \text{ mg}}{\text{g}} \frac{397.2 \text{ g}}{\text{day}} \frac{1}{9.65 \text{ kg}} = 0.10 \text{ mg/kg/day}$$

Results -

Homogeneity Analysis:

Mean of 4 values from the center:

top - 120%

bottom - 114%

Mean of 4 values from the side:

top 123%

bottom - 124%

Stability Analysis:

Percentage of day 0 observed

concentration after 28 days storage:

Top - 98%

Bottom - 100 %

Concentration Analysis:

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. Statistics - Body weight, hematology, clinical chemistry and cholinesterase were analyzed by three-way ANOVA in the following order: time-sex-dose interaction first; then sex-dose interaction; lastly, time-dose interaction. Urine specific gravity, final fasted body weights, and

organ weight data (except gonads) were analyzed by two-way ANOVA for sex and dose interactions. Gonad weights were analyzed by one-way ANOVA. Mean and standard deviations were calculated and Bartlett's test for equality of variances was performed. Data not meeting Bartlett's test criteria were reanalyzed using the common log followed by inverse and square root transformations. The statistical methods used are appropriate.

C. METHODS:

1. Observations:

Animals were inspected daily for signs of toxicity and mortality.

2. Body weight

Animals were weighed weekly.

3. Food consumption and compound intake

Food consumption for each animal was determined and mean daily diet consumption was calculated as g food/animal/day.

4. Ophthalmoscopic examination

Eyes were examined prestudy and at study day 80.

5. Blood was collected by venipuncture from animals fasted overnight. Blood was collected one week before the start of compound administration, 6 weeks into the study, and on day 85 - near study termination. The CHECKED (X) parameters were examined. All cholinesterase levels were determined with a Gilford 203-S Clinical Analyzer. No further information was provided.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		Mean corpusc. HGB conc. (MCHC)
X	Erythrocyte count (RBC)*		Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical Chemistry

ELECTROLYTES		OTHER	
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
ENZYMES		X	Total bilirubin
X	Alkaline phosphatase (ALP)	X	Total serum protein (TP)*
X	Cholinesterase (ChE)	X	Triglycerides
X	Creatine phosphokinase (CK)		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT, ALT)*		
X	Serum aspartate amino-transferase (also SGOT, AST)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis*

Urine was collected from animals by direct aspiration of the urinary bladder at study termination. The CHECKED (X) parameters were examined.

	Appearance	X	Glucose
	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood - occult
X	Sediment (microscopic)		Nitrate
X	Protein	X	Urobilinogen

* Not required for subchronic studies

7. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination [note if not all collected tissues were examined]. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	X	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3
X	Stomach*	X	Lymph nodes*	X	levels) ^T
X	Duodenum*		Spleen*	XX	Pituitary*
X	Jejunum*	X	Thymus*	X	Eyes (optic n.) ^T
X	Ileum*				
X	Cecum*		UROGENITAL		
X	Colon*	XX	Kidneys*+		GLANDULAR
X	Rectum*	X	Urinary bladder*	XX	Adrenal gland*
XX	Liver**	XX	Testes**		Lacrimal gland ^T
X	Gall bladder*	X	Epididymides	X	Mammary gland ^T
X	Pancreas*	X	Prostate		Parathyroids**
			Seminal vesicle	XX	Thyroids**
	RESPIRATORY	XX	Ovaries		
X	Trachea*	X	Uterus*		OTHER
X	Lung*				Bone
	Nose				Skeletal muscle
	Pharynx			X	Skin
	Larynx			X	All gross lesions
					and masses*

* Required for subchronic studies based on Subdivision F Guidelines

* Organ weight required in subchronic and chronic studies.

** Organ weight required for non-rodent studies.

T = required only when toxicity or target organ

II. RESULTS

A. Observations :

Clinical Signs and Mortality - Two females in the 50 mg/kg/day dose group died or were sacrificed moribund. The first was found dead in its cage on the 5th day of treatment. Although the report states that necropsy was performed on this dog, no necropsy report was provided. The cause of death and possible relationship to treatment was not established. The second dog was sacrificed moribund near the termination of the study. This animal displayed severe muscle wasting and inability to raise the hindquarters. The condition of this dog was attributed to decreased food consumption with the possibility that poor palatability of the diet was a factor.

B. Body weight and weight gain: Males and females at the HDT had reduced body weight (reduced 10-18% for both sexes compared to controls) and body weight gain as early as the first month of the study which continued throughout the study. HDT males weighed much less than any other group at the end of the study while HDT females actually lost weight during the course of the study. Table 2 through 5 display these events.

Table 2: Male body weight. All values are given in kilograms.

Dose in mg/kg/day ▽	Prestudy	Day 28	Day 56	Day 91
Control	\bar{x} = 11.09 SD=0.87	\bar{x} =12.23 SD=0.72	\bar{x} = 13.42 SD=0.75	\bar{x} =13.87 SD=0.69
0.1	\bar{x} =10.60 SD=1.29	\bar{x} =11.67 SD=1.42	\bar{x} =12.03 SD=1.47	\bar{x} =12.64 SD=1.51
10	\bar{x} =11.27 SD=1.13	\bar{x} =12.51 SD=1.46	\bar{x} =13.60 SD=1.51	\bar{x} =14.31 SD=1.61
50	\bar{x} =10.72 SD=1.12	\bar{x} =10.97 SD=1.22 (-10%)	\bar{x} =11.17 SD=1.20 (-17%)	\bar{x} =11.36 SD=1.14 (-18%)

Data from current study pages 39 and 40

Highlighted entries are considered toxicologically significant.

Table 3: Female body weight. All values are given in kilograms.

Dose in mg/kg/day ▽	Prestudy	Day 28	Day 56	Day 91
Control	\bar{x} = 8.93 SD=0.79	\bar{x} =9.47 SD=1.53	\bar{x} = 10.15 SD=1.61	\bar{x} =10.52 SD=1.85
0.1	\bar{x} =8.69 SD=0.25	\bar{x} =9.48 SD=0.43	\bar{x} =9.93 SD=0.24	\bar{x} =10.47 SD=0.30
10	\bar{x} =9.06 SD=0.98	\bar{x} =10.12 SD=0.86	\bar{x} =10.77 SD=1.25	\bar{x} =11.46 SD=1.18
50	\bar{x} =8.94 SD=0.81	\bar{x} =8.56 SD=1.02 (-9%)	\bar{x} =8.29 SD=1.52 (-18%)	\bar{x} =8.60 SD=1.47 (-18%)

Data from current study pages 39 and 40

Highlighted entries are considered toxicologically significant.

Table 4: Male % body weight gain.

	Prestudy to Day 28	Day 28 to Day 56	Day 56 to Day 91	Entire Study
Control	\bar{x} =10.3%	\bar{x} =9.8%	\bar{x} =3.3%	\bar{x} =25.1%
0.1 mg/kg	\bar{x} =10.1%	\bar{x} =3.1%	\bar{x} =5.1%	\bar{x} =19.2%
10 mg/kg	\bar{x} =11.0%	\bar{x} =8.7%	\bar{x} =1.1	\bar{x} =26.9%
50 mg/kg	\bar{x} =2.4%	\bar{x} =1.8%	\bar{x} =1.6%	\bar{x} =5.9%

The mean percentages presented in this table were calculated by the reviewer using data from pages 37 and 38.

Table 5: Female % body weight gain.

	Prestudy to Day 28	Day 28 to Day 56	Day 56 to Day 91	Entire study
Control	\bar{x} =6.0%	\bar{x} =7.2%	\bar{x} =3.7%	\bar{x} =17.8%
0.1 mg/kg	\bar{x} =9.0%	\bar{x} =4.7%	\bar{x} =5.5%	\bar{x} =20.4%
10 mg/kg	\bar{x} =11.7%	\bar{x} =6.6%	\bar{x} =6.3%	\bar{x} =26.5%
50 mg/kg	\bar{x} =-4.4%	\bar{x} =-7.3%	\bar{x} =3.8%	\bar{x} =-3.8%

The mean percentages presented in this table were calculated by the reviewer using data from pages 39 and 40.

C. Food consumption and compound intake:

1. Food consumption - Food consumption was determined for each cage of two dogs and individual consumption was not assessed. Food consumption for both sexes of the HDT from the second week of the study onward were much less than any other group. Tables 6 and 7 display food consumption values for weeks 4, 8 and 13. Examination of food consumption values from other weeks would yield similar results. The decreases in the HDT males compared to controls at weeks 4, 8 and 13 are 25%, 10% and 29%, respectively. The decreases at weeks 4, 8, and 13 in the HDT females were 32%, 33% and 32%, respectively.

Table 6: Male food consumption. Values are group means in grams per animals per day for the weeks indicated.

	One week prestudy	Week 4	Week 8	Week 13
Control	\bar{x} =420.4 SD=19.6	\bar{x} =420.6 SD=73.7	\bar{x} = 384.5 SD=71.2	\bar{x} = 449.8 SD=70.9
0.1 mg/kg	\bar{x} =435.6 SD=46.6	\bar{x} =414.1 SD=44.3	\bar{x} =336.4 SD=20.5	\bar{x} =398.5 SD=31.3
10 mg/kg	\bar{x} =473.1 SD=0.3	\bar{x} =441.5 SD=11.1	\bar{x} =474.1 SD=21	\bar{x} =498.7 SD=12.3
50 mg/kg	\bar{x} =459.6 SD=10.2	\bar{x} =313.4 SD=4 (-25%)	\bar{x} =347.9 SD=2.8 (-9%)	\bar{x} =317.7 SD=35.1 (-29%)

Data from current study page 41

Highlighted entries are considered toxicologically significant.

Table 7: Female food consumption. Values are group means in grams per animals per day for the weeks indicated.

	One week prestudy	Week 4	Week 8	Week 13
Control	\bar{x} = 342.7 SD=25.3	\bar{x} =331.4 SD=4.9	\bar{x} =347.3 SD=17.7	\bar{x} = 361 SD=12.9
0.1 mg/kg	\bar{x} =358.8 SD=19.6	\bar{x} =327.1 SD=25.7	\bar{x} =312.3 SD=13.5	\bar{x} =320.7 SD=10.1
10 mg/kg	\bar{x} =387.4 SD=11.2	\bar{x} =343.6 SD=14.8	\bar{x} =361.5 SD=30.1	\bar{x} =358.4 SD=17.6
50 mg/kg	\bar{x} =370.2 SD=22.6	\bar{x} =226.5 SD=35.6 (-35%)	\bar{x} =231.9 SD=49.7 (-33%)	\bar{x} =244.6 SD=N/A* (-32%)

Data from current study page 42

* Not applicable as there were only 2 animals in this group which survived until terminal sacrifice.

Highlighted entries are considered toxicologically significant.

2. Compound consumption - The study does not report compound consumption. The compound was not administered as a constant ppm in the diet. The test material was administered as mg/kg bw by adjusting the concentration of the test material in the diet based the animals previous weeks food consumption and current body weight. Inclusion of details and calculations about how much test article was added to the diet to prepare the diet mixture each week would be desirable in the future.

3. Food efficiency - Food efficiency values were not calculated. Since there was already a marked decrease in food consumption consistent with body weight decrease in the high dose only, actual feed efficiency data is not required. Also, since the food consumption was not assessed for each dog independently, food efficiency calculations would be of limited meaning.

D. Ophthalmoscopic examination - There were no treatment - related findings in the Ophthalmoscopic exam.

E. Blood work:

1. Hematology - At the HDT both sexes showed significant decreases in % PCV (packed cell volume, aka-hematocrit) hemoglobin (Hgb) and RBC (red blood cell counts) at study week 12 but not at midstudy. These three values commonly move in tandem and it is not unexpected that if one value is decreased the other

two would be also. Platelet counts were significantly increased in the HDT of both sexes. These alterations at the HDT were statistical significant at $P < 0.0003$ using a time-dose interaction.

Table 7: Male selected hematology parameters at week 12.
Statistically significant alterations shown in bold*.

	% PCV	Hgb (g/dl)	RBC (10^6)	Plat (10^3)
Control	$\bar{x}=48$ SD=1.1	$\bar{x}=16.3$ SD=0.5	$\bar{x}=6.8$ SD=0.2	$\bar{x}=274$ SD=86
0.1 mg/kg	$\bar{x}=50.7$ SD=3.5	$\bar{x}=16.7$ SD=1.2	$\bar{x}=7.2$ SD=0.5	$\bar{x}=246$ SD=41
10 mg/kg	$\bar{x}=47.4$ SD=3.6	$\bar{x}=15.6$ SD=0.9	$\bar{x}=6.7$ SD=0.5	$\bar{x}=235$ SD=97
50 mg/kg	$\bar{x}=42.6$ SD=2.2	$\bar{x}=14.4$ SD=1.0	$\bar{x}=5.9$ SD=0.3	$\bar{x}=380$ SD=61

Data from current study page 45

* Note that these values were statistically significant using a time-dose interaction evaluation.

Table 7: Female selected hematology parameters at terminal sacrifice. Statistically significant alterations shown in bold*.

	% PCV	Hgb (g/dl)	RBC (10^6)	Plat (10^3)
Control	$\bar{x}=49.9$ SD=1.4	$\bar{x}=16.2$ SD=0.3	$\bar{x}=7.1$ SD=0.3	$\bar{x}=274$ SD=44
0.1 mg/kg	$\bar{x}=51.1$ SD=2.9	$\bar{x}=16.8$ SD=1.1	$\bar{x}=7.2$ SD=0.4	$\bar{x}=263$ SD=40
10 mg/kg	$\bar{x}=51.7$ SD=3.3	$\bar{x}=17.2$ SD=0.9	$\bar{x}=7.3$ SD=0.4	$\bar{x}=277$ SD=27
50 mg/kg	$\bar{x}=40.9$ SD=3.8	$\bar{x}=13.6$ SD=1.3	$\bar{x}=5.6$ SD=0.6	$\bar{x}=457$ SD=143

Data from current study page 48

* Note that these values were statistically significant using a time-dose interaction evaluation.

2. Clinical Chemistry -

Mid-Study

Clinical chemistry values for both sexes of the LDT and MDT were similar to controls. Several parameters at the HDT in both sexes were altered compared to control.

In males - Serum albumin (ALB) was increased 18%, alanine aminotransferase (ALT) was increased 192%, blood urea nitrogen (BUN) was decreased 30%, total protein (TPOT) was decreased 16%, and cholesterol (CHOL) was decreased 21%.

In females - Serum albumin (ALB) was decreased 20.5%, alanine aminotransferase (ALT) was increased 32%, blood urea nitrogen (BUN) was decreased 20%, total protein (TPOT) was decreased 21%, and serum glucose was decreased 16%.

Terminal Sacrifice

Serum ALP levels were significantly increased at the HDT in both sexes. ALB, BUN, TPOT, and CHOL were significantly decreased in both sexes at the HDT. Serum triglycerides (TRIG) were also decreased at the HDT of both sexes, although not significantly. Creatine kinase (CK) levels were increased at the HDT in both sexes, although not significantly. CK levels in the male HDT were 26% more than controls while CK levels in the female were 233% more than controls.

Table 8: Male selected clinical chemistry parameters at terminal sacrifice. Statistically significant alterations shown in bold*.

	ALP U/L	ALB g/dl	BUN mg/dl	TPOT g/dl	CHOL mg/dl	TRIG mg/dl	CK U/L
Con- trol	\bar{x} =59.8 SD=16.7	\bar{x} =3.15 SD=0.06	\bar{x} =16.6 SD=2.2	\bar{x} =5.5 SD=0.2	\bar{x} =218.7 SD=26.2	\bar{x} =49.9 SD=8.8	\bar{x} =104.2 SD=9
0.1 mg/kg	\bar{x} =58.8 SD=22.7	\bar{x} =3.25 SD=0.19	\bar{x} =15 SD=3.1	\bar{x} =5.5 SD=0.3	\bar{x} =212.4 SD=29.5	\bar{x} =54.2 SD=3.5	\bar{x} =103.1 SD=26.6
10 mg/kg	\bar{x} =51.3 SD=10.4	\bar{x} =3.18 SD=0.13	\bar{x} =15.9 SD=3.7	\bar{x} =5.5 SD=0.2	\bar{x} =198.7 SD=35.1	\bar{x} =51.7 SD=10.7	\bar{x} =102.2 SD=24.5
50 mg/kg	\bar{x} = 81.3 SD=16.9	\bar{x} = 2.3 SD=0.27	\bar{x} = 12.5 SD=2.1	\bar{x} = 4.4 SD=0.4	\bar{x} = 134.8 SD=29.4	\bar{x} =38.7 SD=15	\bar{x} =131.3 SD=32.4

Data from current study page 57

* Note that these values were only statistically significant using a time interaction evaluation.

Table 9: Female selected clinical chemistry parameters at terminal sacrifice. Statistically significant alterations shown in bold*.

	ALP U/L	ALB g/dl	BUN mg/dl	TPOT g/dl	CHOL mg/dl	TRIG mg/dl	CK U/L
Con- trol	\bar{x} =61.7 SD=12.8	\bar{x} =3.33 SD=0.15	\bar{x} =13.2 SD=1.8	\bar{x} =5.6 SD=0.3	\bar{x} =193.4 SD=15.6	\bar{x} =59.1 SD=11.3	\bar{x} =103.3 SD=25.8
0.1 mg/kg	\bar{x} =71.1 SD=24.5	\bar{x} =3.38 SD=0.21	\bar{x} =15.3 SD=1.2	\bar{x} =5.6 SD=0.3	\bar{x} =210.1 SD=30.9	\bar{x} =56.7 SD=9.3	\bar{x} =123.1 SD=36.8
10 mg/kg	\bar{x} =84.3 SD=48.7	\bar{x} =3.28 SD=0.17	\bar{x} =14.1 SD=1.1	\bar{x} =5.3 SD=0.2	\bar{x} =206.3 SD=21.2	\bar{x} =60.8 SD=3.6	\bar{x} =124.1 SD=29.3
50 mg/k g	\bar{x} = 105.1 SD=68	\bar{x} = 2.23 SD=0.57	\bar{x} = 12.9 SD=3.9	\bar{x} = 4.3 SD=0.6	\bar{x} = 166.8 SD=30	\bar{x} =50.1 SD=8.4	\bar{x} =343.6# SD=379.5

Data from current study page 60

* Note that these values were statistically significant using a time-dose interaction evaluation.

SD in this group is exceptionally high due to one animal which had a CK at terminal sacrifice 907.2 (histopathology revealed that this animal had skeletal muscular degeneration). The mean without this animal is 155.7 U/L and the SD is 65.2.

3. Cholinesterase - Plasma cholinesterase was statistically significantly reduced at both midstudy and the study's end in the LDT males (11% at midstudy and 0.5% at terminal sacrifice). Plasma cholinesterase was also statistically significantly reduced at midstudy in the LDT females (14%). RBC and brain cholinesterase levels were not affected by compound exposure at the LDT. Midstudy RBC and plasma cholinesterase levels were statistically significantly decreased in both sexes at the MDT (31% and 77% for male RBC and plasma and 18% and 76% for female RBC and plasma). Terminal Brain cholinesterase levels were significantly reduced only at the HDT in both sexes (66% in males and 70% on females). The p values, compared to control, for inhibition of cholinesterase are: Log of plasma 0.0063- LDT; 0.0003 - MDT; 0.0003 - HDT; RBC 0.0072 - MDT; 0.0024 - HDT; Brain 0.0003 - HDT.

Table 10: Male cholinesterase at midstudy and at terminal sacrifice. Statistically significant alterations shown in bold.

	Midstudy- Plasma	Midstudy- RBC	Terminal- Plasma	Terminal- RBC	Terminal- Brain
Control	\bar{x} =1.984 SD=0.087	\bar{x} =2.36 SD=0.169	\bar{x} =1.781 SD=0.168	\bar{x} =1.805 SD=0.175	\bar{x} =4.4 SD=0.65
0.1 mg/kg	\bar{x} = 1.764 SD=0.386 (-11%)	\bar{x} =2.235 SD=0.244	\bar{x} = 1.773 SD=0.535 (-1%)	\bar{x} =2.04 SD=0.121	\bar{x} =4.81 SD=0.61
10 mg/kg	\bar{x} = 0.967 SD=0.151 (-51%)	\bar{x} = 1.69 SD=0.241 (-28%)	\bar{x} = 0.798 SD=0.126 (-55%)	\bar{x} = 1.35 SD=0.109 (-25%)	\bar{x} =4.48 SD=0.24
50 mg/kg	\bar{x} = 0.46 SD=0.116 (-77%)	\bar{x} = 1.63 SD=0.179 (-31%)	\bar{x} = 0.427 SD=0.041 (-76%)	\bar{x} = 1.485 SD=0.271 (-18%)	\bar{x} = 1.48 SD=0.29 (-66%)

Data from current study page 53

Highlighted values are statistically significant.

Table 11: Female cholinesterase values at midstudy and at terminal sacrifice. Statistically significantly altered values are shown in bold.

	Midstudy- Plasma	Midstudy- RBC	Terminal- Plasma	Terminal- RBC	Terminal- Brain
Control	\bar{x} =2.169 SD=0.281	\bar{x} =2.44 SD=0.101	\bar{x} =1.924 SD=0.301	\bar{x} =1.89 SD=0.195	\bar{x} =4.95 SD=0.44
0.1 mg/kg	\bar{x} = 1.873 SD=0.331 (-14%)	\bar{x} =2.415 SD=0.236	\bar{x} = 1.653 SD=0.288 (-14%)	\bar{x} =1.775 SD=0.263	\bar{x} =4.77 SD=0.23
10 mg/kg	\bar{x} = 0.844 SD=0.084 (-61%)	\bar{x} = 1.725 SD=0.111 (-29%)	\bar{x} = 0.822 SD=0.066 (-57%)	\bar{x} = 1.52 SD=0.078 (-20%)	\bar{x} =4.39 SD=0.24
50 mg/kg	\bar{x} = 0.501 SD=0.065 (-77%)	\bar{x} = 1.64 SD=0.247 (-33%)	\bar{x} = 0.478 SD=0.073 (-75%)	\bar{x} = 1.395 SD=0.243 (-26%)	\bar{x} = 1.48 SD=0.31 (-70%)

Data from current study page 54

* Note that these values, with the exception of the brain cholinesterase levels, were statistically significant using a time-dose interaction evaluation. Brain levels significantly decreased by dose alone.

F. Urinalysis - Urinalysis parameters were not altered.

G. Sacrifice and Pathology:

1. Organ weight - relative to controls, the kidney and liver weights, relative to the body weight, were statistically significantly increased at the HDT in both sexes. Absolute liver weights were increased at both sexes at the MDT and HDT, but the increase was not statistically significant. The p values for the increase in relative kidney and liver weights are 0.0093 compared to control for the kidneys (both sexes) and 0.0003 compared to controls for the relative liver (both sexes).

Table 12: Male liver and kidney weights. Values altered statistically significantly are in bold.

	Absolute liver	Relative liver	Absolute kidney	Relative kidney
Control	\bar{x} =387.8 SD=32.2	\bar{x} =2.91 SD=0.11	\bar{x} =55.9 SD=7.29	\bar{x} =0.42 SD=0.06
0.1 mg/kg	\bar{x} =347.9 SD=44.4	\bar{x} =2.833 SD=0.06	\bar{x} =55.69 SD=7.06	\bar{x} =0.46 SD=0.06
10 mg/kg	\bar{x} =432 SD=40.6	\bar{x} =3.14 SD=0.26	\bar{x} =63.56 SD=3.19	\bar{x} =0.47 SD=0.7
50 mg/kg	\bar{x} =430 SD=65.1	\bar{x} = 3.95 SD=0.12	\bar{x} =56.56 SD=4.29	\bar{x} = 0.52 SD=0.3

Data from current study page 61

Table 13: Female liver and kidney weights. Values altered statistically significantly are in bold.

	Absolute liver	Relative liver	Absolute kidney	Relative kidney
Control	\bar{x} =296.5 SD=28.4	\bar{x} =2.963 SD=0.33	\bar{x} =45.0 SD=8.06	\bar{x} =0.45 SD=0.03
0.1 mg/kg	\bar{x} =270.8 SD=29.9	\bar{x} =2.73 SD=0.28	\bar{x} =45.43 SD=3.98	\bar{x} =0.46 SD=0.04
10 mg/kg	\bar{x} =334.6 SD=36.7	\bar{x} =3.12 SD=0.49	\bar{x} =48.93 SD=2.3	\bar{x} =0.46 SD=0.05
50 mg/kg	\bar{x} =321.4 SD=41.5	\bar{x} = 3.90 SD=0.24	\bar{x} =43.46 SD=2.83	\bar{x} = 0.53 SD=0.6

Data from current study page 62

2. Gross pathology - There were very few findings at gross necropsy and the only finding which may have been related to compound exposure was skeletal muscle atrophy seen in 2 HDT females.

3. Microscopic pathology -

a) Non-neoplastic - There were no histopathology findings in the LDT or MDT that were likely related to compound exposure. Proximal tubule vacuolization was seen in 1 MDT female and 2 HDT females. Skeletal muscle degeneration was seen in one HDT female. Hepatic centrilobular hypertrophy was seen in animals of both sexes at the HDT. Thymic atrophy was seen in 3 HDT males.

Table 14: Non-neoplastic histopathology observations.

	Control	0.1 mg/kg	10 mg/kg	50 mg/kg
Kidney-Prox. Tubule Vac.	M=0 F=0	M=0 F=0	M=0 F=1*	M=0 F=2*
Liver-Centri. Hypertrophy	M=0 F=0	M=0 F=0	M=0 F=0	M=3 F=4
Skeletal Muscle-Degeneration	M=0 F=0	M=0 F=0	M=0 F=0	M=0 F=1
Thymus-Atrophy	M=0 F=0	M=0 F=0	M=0 F=0	M=3 F=0

Data from pages 64 to 68.

* Observation at 10 mg/kg was judged to be "very slight"; Both observations at 50 mg/kg were judged to be "slight"

b) Neoplastic - No neoplastic findings of any kind at any dose group were reported.

III. DISCUSSION

A. The study authors considered the proximal tubule vacuolization seen in 1 MDT female and 2 HDT females to be incidental and the thymic atrophy seen in 3 HDT males to be related to stress. Many of the changes seen were attributed by the study authors to be secondary to diet refusal rather than directly related to compound exposure. The decreases in hematocrit hemoglobin, red blood cell counts, blood urea nitrogen, total protein, and cholesterol seen in both sexes at the HDT were attributed to diet refusal. The decrease in relative kidney weight seen in both sexes of the HDT were also attributed to lower body weight resulting from diet refusal. The skeletal muscle atrophy seen in 2 HDT females at gross necropsy and one HDT female at histopathology was attributed to diet refusal. Only the increases in relative liver weight, serum alkaline phosphatase levels, incidence of hepatic centrilobular cellular hypertrophy and cholinesterase inhibitions were considered, by the study authors, to be directly-related to compound exposure.

The reviewer notes that inhibition of cholinesterase was the primary toxic effect of this study. Brain cholinesterase was reduced, compared to controls, at the HDT only in both sexes. Terminal male **brain cholinesterase** was reduced 66.4% and female 71%. **Red blood cell cholinesterase** levels were reduced in both sexes at both the MDT and the HDT. Midstudy males at the MDT and HDT had reductions of 28.4% and 30.9% and midstudy females had reductions of 28.3% and 32.8% (at MDT and HDT respectively). The males at terminal sacrifice had RBC cholinesterase reductions of 25.2% and 17.7% in the MDT and HDT respectively. Females at terminal sacrifice had reductions of 19.6% and 26.2% at the MDT and HDT respectively for RBC cholinesterase. **Plasma cholinesterase** levels were reduced at all dose groups in both sexes. Reduction, compared to controls, for the males at midstudy were: LDT - 11.1%; MDT - 51.3% and HDT - 76.8%. Female midstudy reductions were: LDT - 13.6%; MDT - 61%; HDT - 76.9%. Male reduction at terminal sacrifice: LDT - 0.5%; MDT

- 55.2%; HDT - 76%. Reductions in females at terminal sacrifice were: LDT - 14.1%; MDT - 57.5%; HDT - 75%. All the reductions listed above in both sexes, for all parameters, were statistically significant. However, the plasma cholinesterase inhibitions at the LDT were not considered by the study author, and are not considered by the reviewer to be toxicologically significant. The reductions in plasma cholinesterase, although statistically significant, were never reduced more than 14% in either sex at either midstudy or terminal sacrifice. The reduction in terminal sacrifice males was less than 1%.

Hepatic toxicity is directly due to compound exposure is evident at the HDT of 50 mg/kg/day as indicated by increases in relative liver weight and increased incidence of hepatic centrilobular cellular hypertrophy. The primary toxic effect of compound exposure in this though was cholinesterase inhibition. **The LOAEL in this study is determined to be 10 mg/kg/day based on reductions in plasma and red blood cell cholinesterase measurements. The NOAEL is 0.1 mg/kg/day.** At 50 mg/kg/day, liver toxicity and body weight decreases associated with decreased food consumption are evident.

This study is considered **Acceptable-Guideline** and satisfies the guideline requirement for a subchronic oral toxicity feeding in the dog (870.3151; §82-1 b)

Note Added by the Secondary Reviewer: The above discussion does not include comments on the female dog in the high dose group that died on day 5 of the study. The cause of this dog's death was not determined and there were no comments on any clinical signs that preceded the death and the necropsy report was not provided.

The discussion on poor palatability and related weight effects being the cause of most of the symptoms in the high dose female group as above is one explanation but not a definite conclusion. In general, issues of poor palatability cannot be proven in animal feeding studies although it can be considered as a possible cause. Other factors that may reflect the toxicity of chlorpyrifos methyl may be contributing. Since there is already one other dog that died in the high dose group, questions remain regarding the potential toxicity of chlorpyrifos methyl at the 50 mg/kg/day dose level. The dog chronic feeding study (MRID No.: 099642 and 242154, 1974 study) assessed dose levels of 0, 0.03, 0.1, 1 and 3 mg/kg/day or well below the 50 mg/kg/day dose level where the issue of palatability and possible associated adverse physiological effects are thought to occur. The current study, however, establishes the NOAEL and LOAEL for inhibition of cholinesterase, the primary effect of chlorpyrifos methyl at lower doses, and that a dose of 50 mg/kg/day results in body weight effects (for whatever reason) as well as liver effects. The muscle atrophy and other differences noted may result from the poor palatability and

associated caloric deficiency but direct toxicity of the test material remains a possibility.

- B. Study deficiencies - There were several deficiencies with this study. The study states that the test article -a granular crystal - was mixed directly with the dog chow. Presumably the dog chow was ground into a powder to facilitate mixing and then repelleted following mixing. This information is not provided in the study though it would be desirable to have such information provided. The dogs were fed the dog chow *ad libitum*. It would be preferable to have the dogs fed a set amount rather than *ad libitum*. Food consumption was determined on a per-pen basis. It would be preferable to have individual food consumption values for each dog available. None of these deficiencies are considered sufficient to alter the classification of this study as acceptable-guideline.

Supplement - Range-finding/palatability study (MRID 44668201)

The range-finding/palatability study consisted of a 2-week palatability portion and a 3-day range finding portion. Two female beagle dogs were utilized in the palatability portion of the study. These dogs were feed chlorpyrifos-methyl (95.2%) mixed in their diets - one dog was fed 500 mg/kg/day and the other was fed 1000 mg/kg/day. The dogs were begun on these diets 11/3/87 and on 11/10/87 the doses were reduced to 100 and 250 mg/kg/day. On 11/17/87 the dogs were euthanized and necropsied.

In the range-finding portion of the study one male beagle dog was given a gelatin capsule of chlorpyrifos-methyl (95.2%) at 500 mg/kg on 10/27/87 and given another capsule of 1000 mg/kg the following day, at which point this animal was observed for three days and then necropsied. Another male dog was given a gelatin capsule at 1000 mg/kg on 10/28/87, a capsule at 1500 mg/kg on 10/29/87, and a capsule at 2000 mg/kg on 10/30/87. This dog was then observed for three additional days and then sacrificed and necropsied. Even at the lowest dose of 100 mg/kg food consumption was only 70% of prestudy levels.

The two male dogs in the range finding portion of the study showed no clinical signs other than one episode of vomiting in the lower dose animal and loose stools in the higher dose animal. A slight decrease in body weights of both dogs was observed during the days when the compound was administered, but body weights were back to prestudy levels by sacrifice. There were no findings at gross necropsy.

There were no clinical signs observed in the palatability portion of the study other than transient diarrhea when

presented with the compound containing diets. Both dogs had decreases in body weight during the study; approximately 29% for the high dose dog and 15% for the low dose dog. Food consumption was dramatically reduced in both dogs. Both dogs also showed decreased muscle mass, and decreased mesentery body fat at gross necropsy.

The study author concluded, in regards to the range finding portion of the study, that chlorpyrifos-methyl in gelatin capsules results in transient gastrointestinal distress in dosed dogs. The acute effects are seen at doses at or above 1000 mg/kg. The study author concluded, in regards to the palatability portion of the study, that chlorpyrifos-methyl was unpalatable at 250, 500 and 1000 mg/kg/day and that doses would have to lower than 100 mg/kg/day in order to avoid ill effects from diet refusal.

The reviewer agrees with the conclusions of the study. This study: MRID 44668201; Laboratory study ID TXT:K-046193-028. This study is an acceptable range-finding/palatability study and is classified **Acceptable-nonguideline**. This study should be considered as a supporting study for the study which is the subject of the this DER - MRID 44680601.